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INVESTIGATIONS IN THE RIPENING AND STORAGE OF BARTLETT PEARS[1]

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INTRODUCTION

Physiological studies carried on in connection with the development, ripening, and storage of the Bartlett pear reveal the fact that the factors involved are somewhat different from those connected with the handling of most other fruits. Pears of this variety are not usually allowed to ripen on the tree but are picked as soon as they have attained suitable size for marketing and have reached the condition at which experience has shown they will ripen off the tree without shriveling. The exact tests and sizes that are usually used to determine this degree of development vary somewhat in different sections. Usually no fruit is harvested until it reaches 2¼ to 2⅜ inches in diameter; and such factors as the ease with which the stem separates from the branch, the plumpness of the fruit, or the degree to which the blossom end is smoothly rounded out, the extent to which the sides of the locules or seed cavities have drawn away from the seeds, and the depth to which the tissue crushes when pressed in by the finger are used to determine when the fruit has developed sufficiently to ripen in good condition if removed from the tree. Bartlett pears, when "ripe" off the tree, become soft and full yellow in color. This is the condition referred to by the term "ripe" in this paper.

In the Rogue River district of Oregon a mechanical pressure test (*9*)[3] has been used to some extent during the past year to determine the time of picking, but in the other pear districts of the Pacific coast the methods enumerated above have been followed.

If the pears are left on the trees until they are fully ripe, they are of a very inferior quality. Very often the inside is soft and decayed before

[1] This paper gives the result of a portion of the work carried on under the project "Factors Affecting the Storage Life of Fruit."

[2] The writer wishes to express appreciation to W. S. Ballard, Pathologist, United States Department of Agriculture, for the use of apparatus and for many helpful suggestions.

[3] Reference is made by number (italic) to "Literature cited," pp. 499-500.

the outside becomes yellow; or, if the inside does remain sound, it becomes coarse and granular and has a very inferior texture.

However, in most sections there is a period of from six weeks to over two months between the time at which the first commercial picking is now made and the time the fruit becomes ripe on the tree. Consequently, there is a possibility of considerable variation in the time at which the fruit may be removed from the tree in a green, firm condition and still ripen without shriveling. A consideration of these facts shows the importance of knowing what effect removing the fruit from the tree at varying times has on the keeping quality and on the comparative chemical composition from which the food value and eating quality may be judged.

REVIEW OF LITERATURE

Of much interest in this connection is the work of various investigators who have studied the chemical composition of pears. Some studies have been made of the influence of various environmental factors on the chemical composition of the fruit, which are of sufficient interest to warrant discussing in some detail.

Kulisch (7) concluded, among other things, that the age and shape of the tree and the size of the crops borne have an effect on the composition of the fruit. He found higher sugar content and larger size of fruit correlated in trees that had a light crop as compared to those with a heavy yield. He suggests that with a light crop there is an abundance of carbohydrate material for the full development of the fruit, while a heavy crop tends to draw from other organs of the tree, and even then the crop is cut down in size by an insufficient amount of carbohydrate material.

Ewert (4), in studying the influence on the fruit of the presence of well-developed seeds as compared to parthenogenetic fruit, made analyses of both kinds in several varieties of pears at intervals just previous to and including the time of ripening. In the late fall varieties with which he worked he found a marked increase in sugar as both seeded and seedless pears approached maturity, while the acids appeared to fluctuate somewhat. He found very little starch present in either seeded or seedless fruit. Cane sugar was very rarely present in ripe pears in the varieties studied. He found no very marked and constant differences due to the presence or absence of seeds, the results in this respect apparently varying with the variety.

Kelhofer (6) analyzed the various portions of the fruit of one variety, Siebenmannsbienen, for sugar, acid, and tannin. He found both sugar and acid to be higher in the central flesh portion as compared to the outer or peel region and the inner or core region. The greatest amount of tannin was in the outside or peel region, and there was very little in the core region. Analyses at succeeding dates from time of picking until soft ripe show a slight gain in sugars, a marked loss in acid, and a very marked

loss in tannin material. Analyses of the blossom-end, central, and stem-end portions of the fruit showed slightly more of both sugar and acid in the blossom end, with a slight decrease in the middle and somewhat greater decrease in the stem-end regions.

Ritter (*11*) carried on numerous investigations in the ripening processes of fruit. He found that growing fruit in the dark, so long as the fruit only was darkened, had no effect on the chemical composition; but where the surrounding branches and leaves were kept in darkness or semidarkness, there was a marked reduction, not only in carbohydrates but also in most of the other compounds in the fruit. His figures, however, are based on total grams of the various substances rather than on a percentage basis. He records a progressive increase in the amount of sugar present at successive dates throughout the season and a corresponding decrease in acid. These conclusions are based on work with several varieties of apples and pears.

Riviere and Bailasche (*12*) also record an increase in sugar and a decrease in acid in pears, based on analyses at intervals from June until the fruit is ripe. They found further (*13*) that defoliating spurs decreased the size of the fruit while the defoliation decreased the sugar content and increased the acid content slightly.

Analyses of pears have been made to a limited extent in this country. Dunbar and Bigelow (*3*) determined the acid present in a number of fruits, concluding that in Bartlett, Idaho, Le Conte, and Kieffer pears citric acid predominates, while for all other varieties malic is the main acid present.

Thompson and Whittier (*17*) identified and determined the proportion of the sugars present in a large number of fruits. In Bartlett pears they found levulose to predominate, with some sucrose and a relatively small percentage of glucose. They found, however, that the relative amounts of the various sugars present varied with the state of maturity of the fruit when analyzed.

Recently Cruess and Stone (*2*) made rather detailed studies in connection with Bartlett pear ripening in California. Fruit was secured from several sections of California at intervals during the picking season and was tested for size, soluble solids (Balling test), acids, starch, length of time to ripen from date of picking, and general quality of the ripened product. In general, the later pickings gave a slightly higher Balling reading than the earlier ones, and the same lots gave a somewhat higher reading when ripe than when fresh picked from the tree. The acid test tended to fluctuate a great deal, so much so that it is rather hard to see a correlation between time of picking and acidity. The amount of starch present in the last pickings, as shown by the iodin test, did not seem to be appreciably less than in the earlier pickings. There was a progressive shortening of the time required to ripen the fruit when stored at a constant temperature of 68° F. It was concluded, as a result of a season's work,

that Balling and starch tests were not satisfactory as a means of determining the proper picking conditions for pears.

Considerable work has been done in connection with the storage of Bartlett pears, and the effects of temperatures of storage and the methods of handling are fairly well established. Powell and Fulton (*10*) investigated the effect of storing of Bartlett and Kieffer pears under different temperatures and with different methods of handling. The Bartletts were grown in western New York. The effect of wrapping was tested, and temperatures of 32° and 36° F. were used for storage. Storing immediately, as compared to leaving four days out of storage, was also tried. Fruit stored within 48 hours at 32° kept in prime condition for six weeks, while that delayed four days showed considerable loss in the same length of time. Bartlett pears stored at 32° kept longer and in much better condition than those stored at 36°. Small, well-ventilated packages gave better results than barrels. Wrapped fruit kept in better condition than unwrapped lots. It was found that if the fruit is not too ripe when removed from low temperature storage it will remain sound as long after being removed as will fruit in the same degree of maturity that has not been stored at low temperatures.

Stubenrauch and Ramsey (*15*), working with precooling and storage in the Rogue River Valley of Oregon, picked fruit at three different stages of maturity, packed it, and placed it in a precooling room at 20° F. The room was held at this temperature until the outer fruit in the packages reached 32°; then the room temperature was allowed to rise to 30° or 32°. Their conclusions were that the later picks gave much less physiological decay than the earlier ones, and that by allowing the fruit to remain on the trees fully two weeks longer than was usually done it is possible to hold fruit in storage four weeks at the temperature indicated, if stored promptly, then to ship in iced refrigerator cars and still have the fruit reach the market in good condition. At least 12 to 14 days are required to market fruit from the Rogue River Valley section, if the destination is Atlantic coast cities.

Lewis, Magness, and Cate (*8*) carried on picking and storage investigations in the Rogue River Valley during the summer of 1916. Bartlett pears from three orchards were picked at frequent intervals and the lots divided for the following types of storage: At 70° F. in both humid and dry, or ventilated, storage; at about refrigerator-car temperature, or from 50° to 60°; and in cold storage at 32° and 36°. The results obtained show that in the Rogue River Valley there is a marked increase in size of fruit from week to week even during the picking season, and delaying picking increased the size very markedly. The later pickings were of the highest quality when ripened up. There was a direct correlation between low temperature and length of storage season. A temperature of 32° gave a much longer period during which the fruit remained in good condition than did 36°, while 50° to 60° and 70° gave correspondingly shorter

storage seasons. For storage at 60° or above—that is, common storage—the earliest pickings gave the longest storage season; but when the lower temperature was used the maximum season was obtained in the later picks of more fully matured fruit. This agrees with Stubenrauch and Ramsey in their precooling work. No important correlation could be established between specific gravity of the juice and time of picking, or between starch, as shown by iodin test, and time of picking. Chemical analyses for sugar, acid, and moisture, made under rather unfavorable conditions, were also rather conflicting in results but showed a tendency toward an increase in sugar as the season advanced.

Further work (*16*) carried on in the Rogue River region gave further detailed evidence of marked increase in size of the fruit during the picking season. The influence of time of picking and temperature of storage upon keeping quality correlated closely with that obtained the year before. A "pressure test," or the measure of the amount of pressure necessary to make a depression of certain size in a pear at various stages of development and maturity was followed through the season; and a marked correlation was established between the time of picking and the resistance of the fruit to pressure, the resistance growing less the longer the fruit remained on the tree.

Some work has been done recently on the effect of storing Bartlett pears at high temperature. Shamel (*14*) placed a box of pears in a lemon-curing room, held at a temperature of about 90° F. and at a humidity averaging 85° to 90°. The pears kept perfectly and without ripening for a month. Upon removal from the storage they ripened normally and were of good quality. Shamel attributed these results to the high humidity.

Taylor and Overholzer (*16*), following Shamel's work, stored small lots of Bartletts at temperatures ranging from 69° to 104° F., with one lot at 32° storage as a control. High humidity and normal dryness of air were compared at each temperature. Fruit held at 69° to 85° ripened most quickly. When the storage temperature was above 85°, the ripening of the fruit was retarded. Fruit stored at 104° was two to three weeks later in ripening than that stored at 85°. Humidity had no effect other than that of preventing shriveling at the high temperatures.

From a summary of all the storage work that has been done on Bartlett pears it is apparent that the lower the temperature used, down to 31° or 32° F.—the lowest temperatures of storage used in these experiments—the longer the storage season will be. Most rapid ripening is attained at a temperature of 70° to 80°, while either higher or lower temperatures tend to retard ripening. It is not definitely known just why these higher temperatures should retard ripening, but it is of interest to note that many of the processes concerned with the ripening of fruit are chemical reactions brought about by enzym action. It is well known that there are minimum, optimum, and maximum temperatures for enzym

action; and it is an interesting possibility that the temperatures which retard ripening may be sufficient to inhibit enzyms. It is well known that plant growth, in which many of the processes are similar to those in the ripening fruit, is inhibited by high temperatures.

From the foregoing summary of the work that has been done on pear ripening and storage it is apparent that for the varieties tested there has generally been found an increase in sugars as the season advanced. The data regarding acid are rather more conflicting. The work on storage and time of picking of Bartletts has shown that the later pickings have given a longer low temperature storage season and a higher quality in the ripened product.

In this investigation it has been the purpose to make a careful study of the changes that take place in Bartlett pears from the Pacific coast regions during the time they are developing, including the commercial picking season and extending somewhat beyond it. The effect of the time of removing the fruit from the tree on its content of acid, sugar, starch, and moisture has been studied. It has also been the purpose to determine the changes that take place in the fruit between the time of picking from the tree and the time the fruit is in prime eating condition—that is, soft and full yellow ripe—and to see if the temperature at which the fruit is held during ripening has any appreciable effect upon its composition.

The principal part of the work was carried on with fruit from two important pear sections of California. One lot was secured from an orchard at Sacramento. This orchard is typical of the large Sacramento River pear district and is grown on reclaimed, irrigated soil adjacent to the river. The summer here is warm and dry, but abundant water is available for irrigation.

Fruit from a ranch near Suisun, Calif., was also used. This section is slightly higher and nearer the coast than the Sacramento district. Fruit from this section is quite representative of much of the central California pear region away from the Sacramento River. Fruit from both of these orchards was picked at frequent intervals from early June, almost a month before commercial picking started, until after the close of the shipping season.

For purposes of comparison, fruit was secured from Medford, Oreg. This is representative of well-grown fruit on heavy soil in a typical irrigated Rogue River Valley orchard. Three boxes were secured from this orchard, one picked July 19, about 18 days before commercial picking started, one August 8, representing the beginning of the shipping season, and one August 28, at the end of the shipping season for Bartletts.

Fruit was also secured from the Selah section of the Yakima Valley, Wash. The first fruit from this section was picked July 28, 1919, followed by a shipment August 13, at the beginning of the shipping

season. Fruit picked August 23 was representative of the late shipments. Because of trouble from the fruit breaking down while in transit in former years, shippers from this section endeavored to reduce the later shipments to a minimum, and much of the late fruit was marketed through the canneries. This accounts for the relatively short shipping season in this section.

The fruit was picked, packed, and shipped by express to the laboratory at Watsonville, Calif. Fruit from Suisun usually arrived in 1 day, from Sacramento in 1½ days, from Medford, Oreg., in 2 days, and from Selah, Wash., in 4 days. Consequently, a somewhat longer time elapsed between the time of picking and time of storing the Washington pears than was the case with other districts.

As soon as the fruit arrived at the laboratory, each picking was divided into four lots. One lot was sampled for immediate analysis, while the remaining three were placed in storage until ripe. One of these was held at from 65° to 70° F., approaching the temperature at which pears ripen most quickly. One was stored at a fluctuating temperature of 34° to 50°, averaging a little above 40°. This is not far from representative of conditions in an iced refrigerator car in transit, although at a slightly lower temperature. The third lot was held at a temperature ranging from 28° to 32° and representing about the minimum temperature at which the pears could be held without the formation of ice in the fruit. The average temperature was slightly below 30°, though some of the time it was down to 28°, with no apparent bad results. It was impossible to allow the fruit to reach full maturity in this storage because of the length of time it would require, so part of all lots was removed October 14, after being from 1½ to 3½ months in storage. It was allowed to ripen at laboratory temperature and was analyzed.

In this way it has been possible to get a comparison between fruit when it is fresh picked from the tree and the same lot of fruit when ripened at temperatures approximating 70°, 40°, and 30° F. In planning the work it was not the thought to develop the storage phase primarily, but it has been possible to compare the length of the storage season with results attained by other investigators.

ANALYTICAL METHODS

SAMPLING.—A sample comprising portions of 15 pears was used for each lot. The fruit was first halved longitudinally and then a section from two opposite sides was removed, the cut being made from the core outward so that a fair proportion of the tissue from all the different regions of the fruit was secured. Any adhering portions of core were removed, and the peel was taken off with as little of the fleshy portion as possible. Sections of sufficient size were taken from each of the 15 pears to make a composite sample of over 300 gm.

The whole sample was then run through a sampling press, constructed on the principle described by Clark (*16*). In this press the tissue was forced through fine perforations which left it in a finely divided state. It was then very thoroughly mixed by stirring and carefully weighed out in six 50-gm. portions. By this method there is a very slight loss in moisture due to evaporation; but by working as rapidly as possible after the sample is run through the press this loss is reduced to a minimum and is closely comparable in all cases. The method of sampling employed was very satisfactory from the standpoint of giving close checks on such duplications as were run.

SUGARS AND ACID-HYDROLYZABLE REDUCING MATERIAL.—Duplicate samples were prepared for these determinations, but only one was run through. The 50-gm. samples were covered with about 100 cc. of 95 per cent alcohol, 3 to 5 drops of ammonia were added to neutralize the acid, then the samples were immediately put on the steam bath and boiled vigorously for a few minutes. Most of the excess ammonia was driven off by the boiling. Preliminary tests of glucose solution treated in this manner with dilute ammonia showed no measurable breaking down of the sugar. The alcohol was then filtered off through an alundum thimble, and the sample was extracted with alcohol in a Soxhlet extractor for about 12 hours. The extract was then added to the original alcohol filtrate, and the sample was made up to 500 cc. with distilled water. Fifty cc. of this were cleared with neutral lead acetate and were made up to 250 cc. The excess lead was removed, and the sugars were determined in 20 cc. of the cleared solution, according to Mathews's[1] modification of Munsen and Walker's and Bertrand's methods. Duplicate titrations with permanganate solution were checked to within 0.2 cc.

Fifty cc. of the cleared solution were inverted in 3.5 per cent hydrochloric-acid solution, standing about 20 hours at room temperature. The total reducing substances were then determined in this solution.

The residue in the thimble from the Soxhlet extraction was removed quantitatively, was dried, and 125 cc. of 2.5 per cent hydrochloric acid were added for hydrolysis by the modified Sachsse method.[2] Reducing substances were determined as outlined above and were figured as dextrose. This alcohol-insoluble acid-hydrolizable material probably consists mainly of starch, galactans, hemi-celluloses, and pectin in the green fruit fresh from the tree. In the ripe fruit there is no starch present, as shown by the iodin test, and the reducing material must come almost entirely from such sources as the galactans, hemicellulosses, and pectins. Much of the reducing material

[1] MATHEWS, A. P., PHYSIOLOGICAL CHEMISTRY... ed. 2, p. 994. New York, 1916.

[2] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 53. 1908.

formed may be pentoses, but calculating all reducing substances as dextrose gives a satisfactory comparison.

ACID.—Two 50-gm. portions of the sample were weighed into beakers, about 150 cc. of distilled water were added, and this was immediately boiled to render the cells permeable. After the portions cooled they were made up to 500 cc., 2 cc. of toluol were added to each as a preservative, and they were allowed to stand with frequent shaking for 3 days. One hundred cc. of the supernatant liquid were then drawn off for titration with *N*/10 sodium hydroxid. Duplicate samples made by this method checked very closely.

DRY WEIGHTS.—Two 50-gm. samples were weighed directly into evaporating dishes. These were then dried down on the steam bath sufficiently to prevent growth of microorganisms; then when a sufficient number accumulated, they were put in a vacuum oven at 70° F., dried five days, removed and weighed, then returned to the oven for two days. During the last two days the decrease in weight was about 50 mgm.; but, since all lots were run in exactly the same manner, the results are closely comparable. All dry weight determinations were made in duplicate, and the figures presented are averages. While considerable variation occurs in successive determinations, duplicates in all cases checked very closely.

PRESENTATION OF DATA

The results of all the analyses are summarized in Tables I to IV. Table I includes all the data of fruit from the Sacramento orchard, Table II those from the Willota Orchard at Suisun, Calif., Table III those from Medford, Oreg., and Table IV those from Yakima, Wash.

TABLE I.—*Chemical analyses of Bartlett pears from Sacramento River Valley, Calif., in 1919*

Lot No.	Date of picking.	Acid as malic.				Reducing sugar as glucose.				Total sugar as glucose.				Alcohol-insoluble, acid-hydrolyzable substances as glucose.				Dry weight.			
		Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.		
			70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.
		P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1	June 12	0.3442	0.2561	0.2902	......	3.70	6.28	5.11	......	4.40	7.15	5.61	......	3.76	2.15	2.37	......	16.60	16.93	15.86	......
2	18	.3380	.3032	.2869	......	3.86	6.55	5.03	......	4.32	7.40	6.13	......	3.85	1.94	1.96	......	16.57	15.87	14.99	......
3	July 5	.3606	.3899	.3198	0.2640	5.40	7.43	6.31	6.50	6.00	8.37	7.00	7.77	3.77	1.67	1.76	1.56	16.03	15.72	15.20	16.07
4	12	.3319	.4084	.3334	.2514	5.53	7.43	6.50	6.56	6.07	8.34	7.05	8.07	3.40	1.71	1.99	1.81	16.17	15.71	15.94	16.87
5	23	.3258	.3954	.3278	.2897	6.20	7.39	6.40	6.60	6.65	8.87	7.33	8.22	2.73	1.56	1.87	1.52	16.02	15.98	15.54	15.99
6	Aug. 13	.2568	.2630	.2554	.2394	7.12	7.64	7.70	7.56	8.35	10.10	9.40	9.72	2.36	1.20	1.24	1.33	17.37	15.98	17.15	17.18

TABLE II.—*Chemical analyses of Bartlett pears from Suisun, Calif., in 1919*

Lot No.	Date of picking.	Acid as malic.				Reducing sugar as glucose.				Total sugar as glucose.				Alcohol-insoluble, acid-hydrolyzable substances as glucose.				Dry weight.			
		Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.		
			70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.
		P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1	June 10	0.4288	0.3176	0.3215	......	2.48	4.55	3.62	......	3.20	5.05	4.04	......	3.87	2.95	3.05	......	16.08	17.58	17.72	......
2	July 1	.3100	.3106	.2264	0.2377	4.79	7.01	4.87	6.10	5.20	7.67	5.58	7.20	3.70	2.13	1.76	2.00	15.56	16.39	14.18	16.06
3	10	.2880	.2950	......	......	5.75	8.05	......	......	6.37	8.90	......	......	3.66	1.99	......	......	17.63	18.08	......	......
4	22	.2492	.2670	.2148	.1963	6.34	7.45	6.45	6.44	6.90	9.05	7.70	7.73	2.79	1.56	1.59	1.54	16.61	16.46	15.83	16.47
5	Aug. 6	.2301	.2486	.2107	.2024	6.78	7.63	6.95	7.75	8.22	9.98	8.67	9.20	1.94	1.04	1.18	1.23	15.50	17.31	15.40	16.20

TABLE III.—*Chemical analyses of Bartlett pears from Rogue River Valley, Oreg., in 1919*

Lot No.	Date of picking.	Acid as malic.				Reducing sugar as glucose.				Total sugar as glucose.				Alcohol-insoluble, acid-hydrolyzable substances as glucose.				Dry weight.			
		Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.		
			70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	July 19	0.3249	0.3908	0.3037	0.2798	5.59	7.39	6.38	6.60	6.07	8.19	7.15	7.60	3.20	1.55	1.64	......	15.50	15.64	......	15.91
2	Aug. 8	.3263	.3639	.3358	.3054	6.29	7.41	6.45	7.41	6.95	8.71	7.33	8.72	2.43	1.03	1.41	1.43	15.27	17.29	14.61	16.10
3	28	.3570	.3631	.3516	.3591	......	7.95	7.87	7.98	......	10.10	9.35	10.00	......	1.35	1.44	1.43	17.28	17.70	17.34	17.95

TABLE IV.—*Chemical analyses of Bartlett pears from Yakima Valley, Wash., in 1919*

Lot No.	Date of picking.	Acid as malic.				Reducing sugar as glucose.				Total sugar as glucose.				Alcohol-insoluble, acid-hydrolyzable substances as glucose.				Dry weight.			
		Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.			Green fruit.	Same lot ripe after storage.		
			70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.		70° F.	40° F.	30° F.
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
	July 28	0.2790	0.2215	......	0.2385	4.95	6.28	......	5.65	5.28	6.95	......	6.50	2.44	1.24	......	1.51	14.10	14.40	......	14.22
	Aug. 13	.3510	.3800	0.3335	.2852	6.35	......	6.74	6.86	6.80	......	7.70	8.20	2.65	1.29	1.49	1.49	17.01	15.70	15.05	15.76
	23	......	.4435	.4510	.4360	......	7.43	7.50	6.31	......	9.07	8.65	9.35	......	1.29	1.38	1.30	......	16.88	17.02	17.24

For purposes of comparison and discussion, however, the results are also presented as a series of curves, in which it is possible to bring similar substances under the varied treatments into direct comparison.

INFLUENCE OF TIME OF PICKING UPON SUGAR CONTENT OF FRUIT

Figures 1 to 4, inclusive, summarize the data on the development of sugars in the fruit at the various intervals at which pickings were made

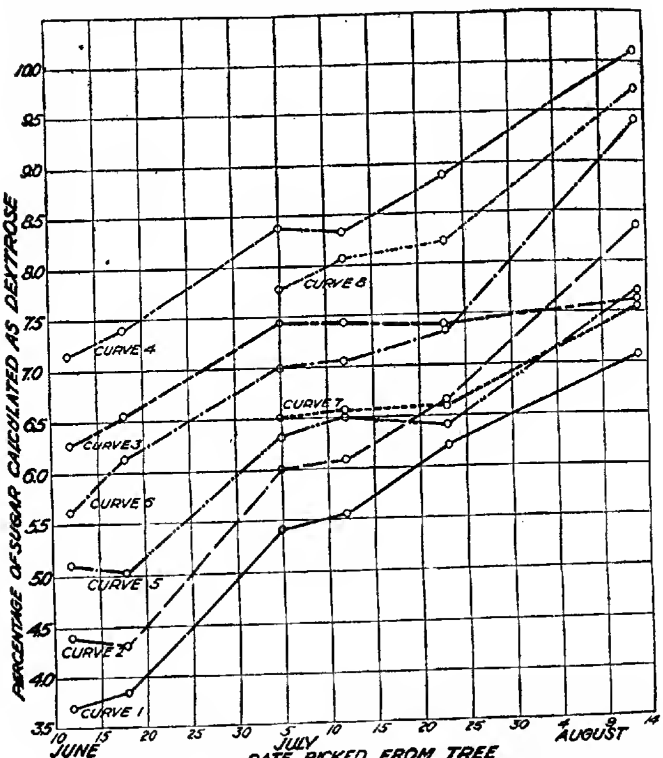

FIG. 1.—Sugars in Bartlett pears from Sacramento, Calif.: Curve 1, reducing sugars in green fruit when picked from the tree; curve 2, total sugars in green fruit when picked from the tree; curve 3, reducing sugars in collateral lots after ripening at 70° F.; curve 4, total sugars in collateral lots after ripening at 70°; curve 5, reducing sugars in collateral lots after ripening at 40°; curve 6, total sugars in collateral lots after ripening at 40°; curve 7, reducing sugars in collateral lots after ripening at 30°; curve 8, total sugars in collateral lots after ripening at 30°.

and the influence of various types of storage upon the sugar content of the fruit picked at these same intervals. Curve 1 in each figure represents the reducing material present, figured to percentage of green weight in the fruit fresh picked from the tree. According to Thompson

and Whittier (17) this consists mainly of levulose, but it has been figured as dextrose here because comparative results are of primary interest. Curve 2 represents the total sugar or reducing material after inversion, so that the distance between curves 1 and 2 represents the amount of

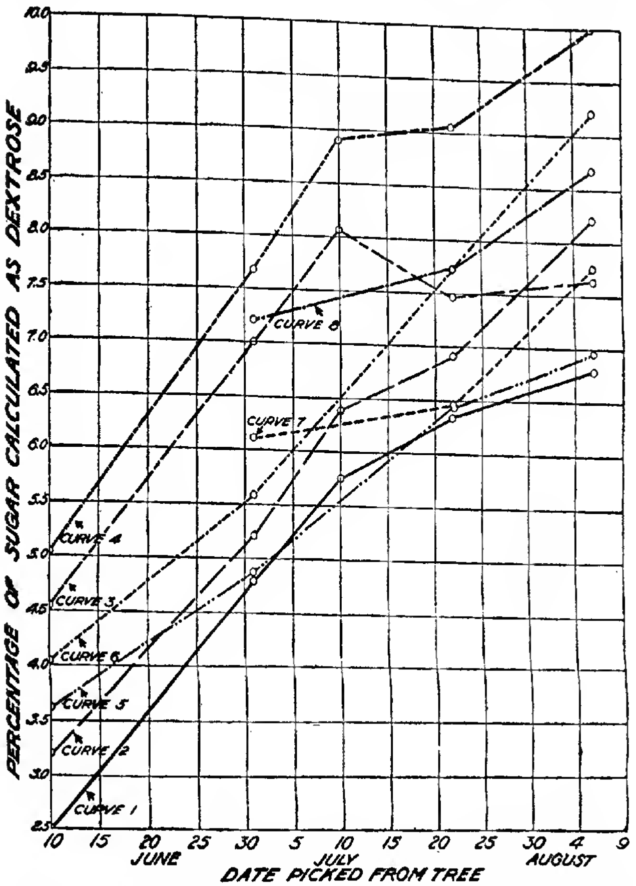

FIG. 2.—Sugars in Bartlett pears from Suisun, Calif.: Curve 1, reducing sugars in green fruit when picked from the tree; curve 2, total sugars in green fruit when picked from the tree; curve 3, reducing sugars in collateral lots after ripening at 70° F.; curve 4, total sugars in collateral lots after ripening at 70°; curve 5, reducing sugars in collateral lots after ripening at 40°; curve 6, total sugars in collateral lots after ripening at 40°; curve 7, reducing sugars in collateral lots after ripening at 30°; curve 8, total sugars in collateral lots after ripening at 30°.

sucrose present. There is in every case a marked increase in the amount of reducing sugar present at successive dates of picking. This increase is somewhat more rapid early in the season, although a distinct increase occurs as long as any pickings are made. It is unfortunate that it was

impossible to secure even later pickings to see if this increase in reducing sugar continues until the fruit is fully ripe on the tree.

The amount of sucrose remains nearly constant at less than 1 per cent throughout the early season. In the late season there was an increase in the amount of sucrose to 1½ per cent in the late picks of California

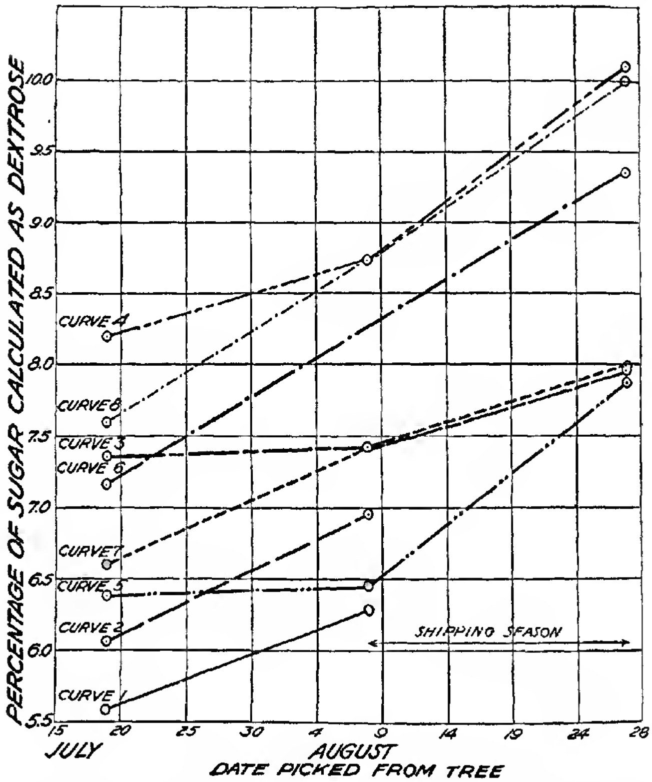

FIG. 3.—Sugars in Bartlett pears from Medford, Oreg.: Curve 1, reducing sugars in green fruit when picked from the tree; curve 2, total sugars in green fruit when picked from the tree; curve 3, reducing sugars in collateral lots after ripening at 70° F.; curve 4, total sugars in collateral lots after ripening at 70°; curve 5, reducing sugars in collateral lots after ripening at 40°; curve 6, total sugars in collateral lots after ripening at 40°; curve 7, reducing sugars in collateral lots after ripening at 30°; curve 8, total sugars in collateral lots after ripening at 30°.

fruit. The increase in sucrose in the late pickings is such that the increase in total sugar shows no falling off in rate up to the date of the last pickings secured. The less rapid increase in reducing sugar is counteracted by the increase in sucrose.

Curves 3 and 4 in each figure represent reducing and total sugar, respectively, when the fruit reached prime eating condition in a storage

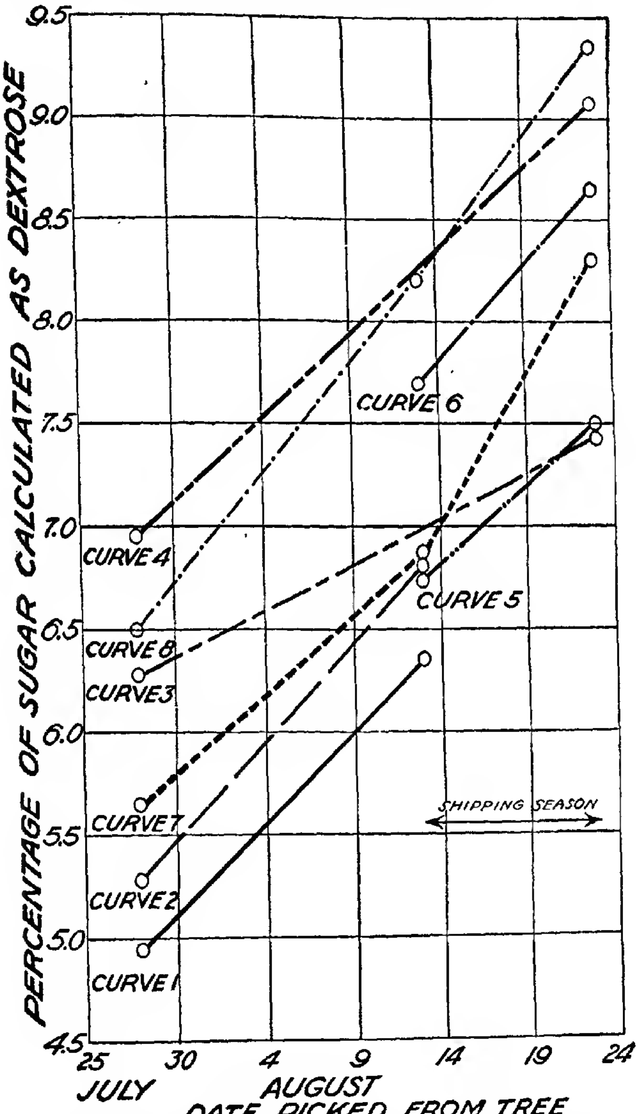

FIG. 4.—Sugars in Bartlett pears from Yakima, Wash.: Curve 1, reducing sugars in green fruit when picked from the tree; curve 2, total sugars in green fruit when picked from the tree; curve 3, reducing sugars in collateral lots after ripening at 70° F.; curve 4, total sugars in collateral lots after ripening at 70°; curve 5, reducing sugars in collateral lots after ripening at 40°; curve 6, total sugars in collateral lots after ripening at 40°; curve 7, reducing sugars in collateral lots after ripening at 30°; curve 8, total sugars in collateral lots after [illegible]

temperature from 65° to 70° F., curves 5 and 6 in 40° storage, and curves 7 and 8 in 30° storage. The distance between these curves and curves 1 and 2, at the various dates, represents the increase in sugar as the fruit ripened in the different storages. It will be noted that the sugar runs uniformly highest in the fruit ripened at 70°. This is true for both total and reducing sugar in fruit picked at all the different dates. Apparently, either the loss of sugar from respiration is less, or more substances insoluble or nonreducing in the green fruit are changed to soluble reducing material when the fruit is ripened at this optimum ripening temperature than when ripened at lower temperatures.

In every lot, regardless of the section from which it came or the date at which it was picked, fruit held at 30° F. was higher in sugar than that stored until ripe at 40°. It must be borne in mind, however, that the 30° fruit was not completely ripened in storage but was held for periods of from a little over three months in the early picked lots to a little over six weeks for the last lots from Oregon and Washington. Then it was removed and held at warm room temperature until ripe, the time required beiug four to six days. This may have made some difference in the analytical results. Also, at one period, because of a sudden drop in temperature, the fruit picked in the earlier lots was partly frozen. It was thawed very gradually, and no ill effects of the freezing were noticeable afterwards. The fact that the frozen lots and those of the later pickings that did not freeze showed no marked difference in analyses other than that to be expected from the results with the same lots in other storages is also evidence that the carbohydrates of the fruit were not materially affected by the freezing.

The general effect of storage upon the sugar content of the fruit was very similar, however, in fruit from the different sections. The curves for total sugar—No. 2, 4, 6, and 7—cross in only one point in all the figures, showing that the relative amounts of sugar in the different storages run the same in all cases. It seems well established, therefore, that the highest amount of sugar will be secured by holding the fruit at optimum temperature for ripening. In case it is necessary to prolong the time of keeping the fruit, holding it at very low temperature until near the time it is needed and then ripening it up at optimum temperature gives a higher sugar content than holding it at a temperature just low enough to retard the ripening processes. From the results obtained by Gore (5) on the respiration activity of fruits at different temperatures, it would be expected that respiration would occur at least three times as rapidly in the 70° as in the 40° F. The number of days required to ripen the fruit in the two storages was about in the proportion of three days at 40° to one at 70°, so the total respiration activity would seem to be about equal. If this is true, it would seem that certain factors other than respiration must enter into the relative amounts of sugar present in the different storage lots.

RELATION OF SUCROSE TO REDUCING SUGAR DURING STORAGE

There is a marked increase in sucrose during the time between picking and the full ripening of the same fruit. This is shown by a comparison of the distance between curves 1 and 2, representing the sucrose in the fruit fresh from the tree, and between curves 3 and 4, showing the sucrose in the same fruit when ripe. There is a very marked increase in sucrose during storage in the earlier pickings, and this increase is even more marked in the late pickings. The late pickings show very little increase in reducing sugar between the time of picking and the time the fruit was ripened, while the increase in sucrose was very marked, being sufficient to make the total sugar increase between the time of picking and full ripeness practically as much in late-picked as in early picked fruit. There seems to be little relation between temperature and kind of sugar in the fruit, the 70°, 40°, and 30° F. storage lots being quite similar in the proportion of sucrose to reducing sugar.

A review of all the curves indicates that, whereas in the early picked fruit almost all of the sugar is in the form of reducing substances, the increase in reducing sugars in successive lots, as the season progresses, is much less marked than is the increase of sucrose. In all the lots, reducing sugar in the late picks seemed to run to between 7 and 8 per cent of the green weight of the fruit, after which there was very little increase in reducing substances, while sucrose continued to increase rapidly until after the last pickings were made.

RELATION OF ACIDITY TO TIME OF PICKING

In the relation of acidity to the time of picking there is not so distinct a correlation in all cases as there is for the sugars. Fruit from different districts seemed to respond somewhat differently in this regard, though certain general tendencies hold for all regions. Figures 5 to 8 summarize the results on acidity, computed as malic acid in terms of percentage of wet weight of the fruit. Curve 1 in each plot represents acid in the green fruit, curve 2 in fruit ripened at 70° F., curve 3 in fruit ripened at 40°, and curve 4 in fruit ripened at 30°.

In fruit from Suisun, Calif., (fig. 6) there is a constant decrease in acid in the green fruit from the time of the first picking until the last. On the other hand, in fruit from Sacramento (fig. 5) there is a slight rise until July 5, about the opening of the picking season, followed by a drop toward the end of the season. In fruit from the more northern sections, however, there is an increase in acid instead of a decrease. The increase is rather slight in the Medford pears (fig. 7), but very marked in those from Yakima (fig. 8). It is interesting to note that, whereas the acidity of the fruit decreased in the California sections, fruit from the Medford section showed a slight increase, and that from the still more northern Yakima section showed a very marked increase

as the season advanced. While the data are much too limited to justify the assumption that this relation of acidity to latitude generally holds, the results of the one year's work are of sufficient interest to warrant further study along this line.

EFFECT OF STORAGE UPON ACIDITY

A somewhat greater uniformity exists in the relation of temperature of storage to the acidity of the ripened product than was found in connection with the time of picking. In the first place, it will be noted

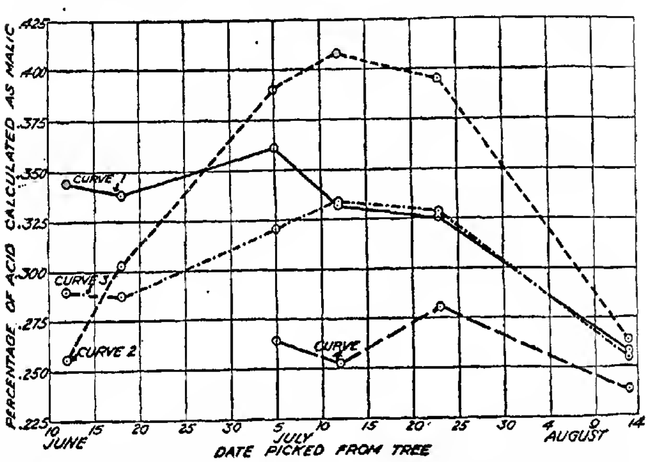

FIG. 5.—Acids in Bartlett pears from Sacramento, Calif.: Curve 1, acid in green fruit when picked from the tree; curve 2, acid in collateral lots after ripening at 70° F; curve 3, acid in collateral lots after ripening at 40°; curve 4, acid in collateral lots after ripening at 30°.

that in the early picks there is a wide variation in amount of acid, due to the temperature of the storage used; and in most cases there is a greater amount of acid in the green fruit than in the ripened fruit, regardless of the temperature at which it was held. Fruit picked in a very immature condition has less acid when ripened than at the time of picking. (Curves 1–4, fig. 5–6, early pickings.)

Fruit picked at about the time of the opening of the commercial season, however, behaved somewhat differently. In every case the fruit ripened at 70° F. contained a higher percentage of titratable acid than did the same fruit when picked from the tree. This is of interest especially in connection with the question of whether fruit acids are synthesized in the fruit itself or whether they are carried to the fruit from the leaves. The fact that there is an increase in the acid between the time the fruit is removed from the tree and the time of its becoming ripe is evidence that there is an actual synthesis of acid in the fruit itself.

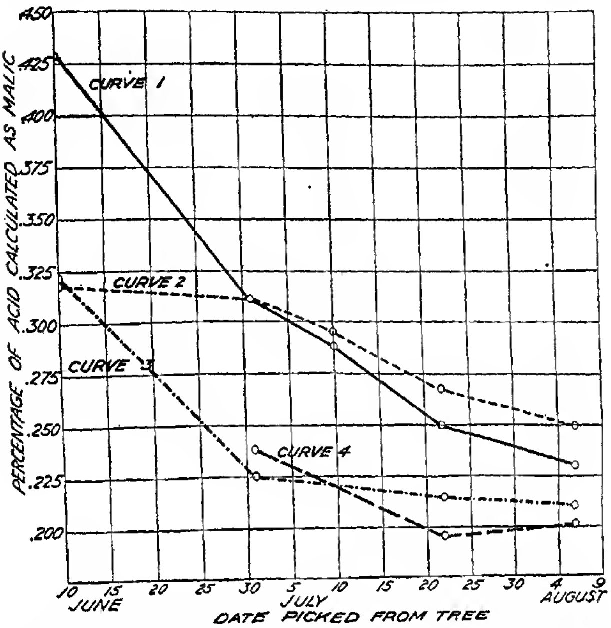

Fig. 6.—Acids in Bartlett pears from Suisun, Calif.: Curve 1, acid in green fruit when picked from the tree; curve 2, acid in collateral lots after ripening at 70° F.; curve 3, acid in collateral lots after ripening at 40°; curve 4, acid in collateral lots after ripening at 30°.

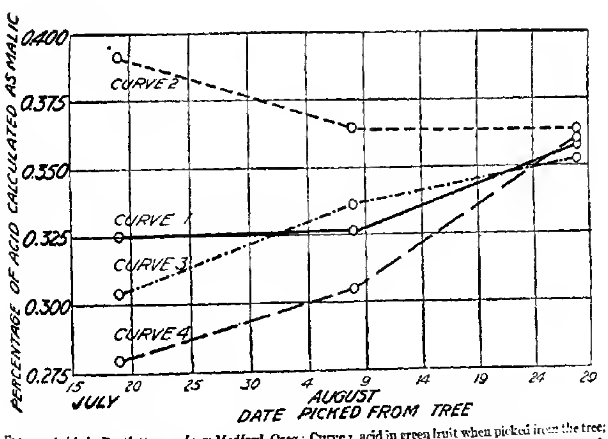

Fig. 7.—Acids in Bartlett pears from Medford, Oreg.: Curve 1, acid in green fruit when picked from the tree; curve 2, acid in collateral lots after ripening at 70° F.; curve 3, acid in collateral lots after ripening at 40°; curve 4, acid in collateral lots after ripening at 30°.

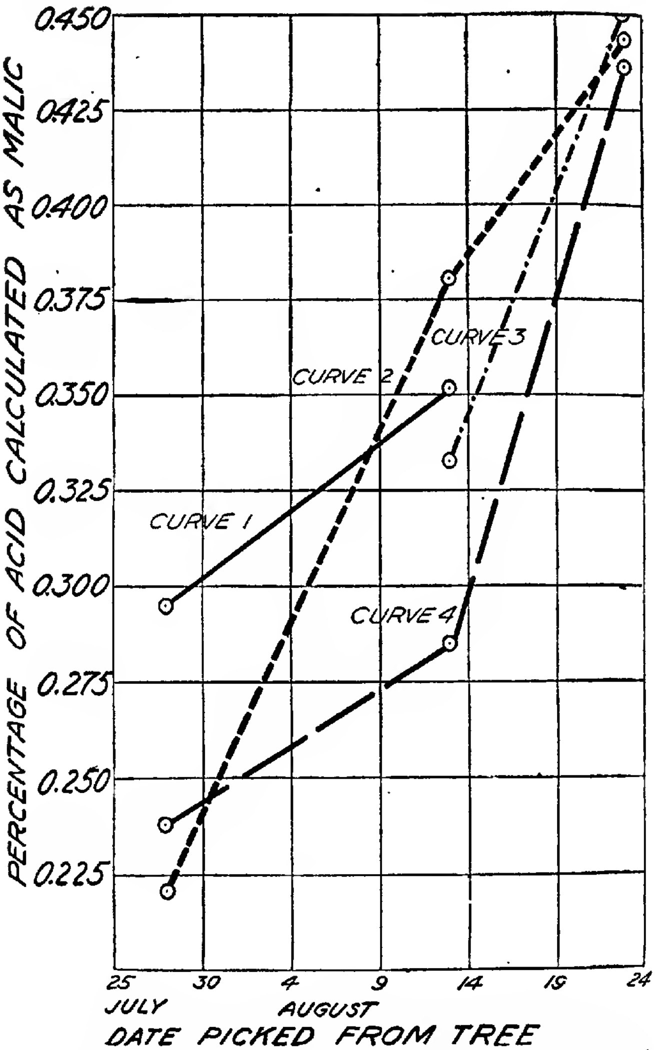

FIG. 8.—Acids in Bartlett pears from Yakima, Wash.: Curve 1, acid in green fruit when picked from the tree; curve 2, acid in collateral lots after ripening at 70° F.; curve 3, acid in collateral lots after ripening at 40°; curve 4, acid in collateral lots after ripening at 30°.

It was almost invariably true that fruit held at 30° F. had a lower acid content than that held in either 40° or 70° storage. This is especially interesting in connection with the fact that the sugars were much higher in the 30° storage lots than in the 40° pears. (Curves 2, 3, 4, fig. 5–8.)

In the latest picked lots from all sections the acid was very nearly the same, both in the green fruit and in the ripened fruit, regardless of the temperature of storage used. The acid content seems to become more nearly stabilized in the late season. It will be noted, however, that whereas the acid content in the California pears was very low at this time, in the fruit from the northern regions it was higher than at any other time during the season.

The question as to why the acid content should remain more nearly constant in late-picked than in early picked fruit is naturally suggested by these results, but until something more is known of the synthesis of the acids and the rôle they play in fruit and plant respiration a solution seems improbable.

ALCOHOL-INSOLUBLE, ACID-HYDROLYZABLE REDUCING SUBSTANCES

Results of analyses made as these were, by hydrolyzing the residue from an alcohol extraction with dilute acid and determining the reducing substances present, have usually been reported as starch. That this may be very misleading is shown by the fact that ripe pears contain no starch, as proved by iodin tests, yet the residue from the alcohol extraction after being hydrolized contained a considerable amount of reducing material. It is almost certain that an equal or even greater amount of such material found in the green fruit is also made up of substances other than starch. For this reason the percentage weight of this group of substances has been figured as dextrose, and the figure includes starch, together with certain hemicelluloses, galactans, pectin materials, etc., which may be present in varying amounts.

A study of the data presented in Tables I to IV shows that these reducing substances run highest in the earliest lots when first picked from the tree. There is a decreasing amount in the green fruit at successive pickings, until the last lots contain only about two-thirds as high a percentage of these substances as do the earliest pickings.

There is a very marked drop in the amount of alcohol-insoluble, acid-hydrolyzable reducing substances present in the storage-ripe fruit as compared to the similar lots when picked. Compare column 15, green, with columns 16 and 18, ripe. This, of course, is natural, since all the starch and probably some of the other material have disappeared. It is interesting to note, however, that there is also a decrease in the amount of these reducing substances in the ripe fruit from late pickings as compared to ripe fruit from early pickings. This decrease in many cases amounts to 50 per cent of the total and seems to indicate that as the fruit develops on the tree much material other than starch changes over to sugar or is in condition to change over after picking.

These results are interesting when considered in connection with the findings of Lewis, Murneek, and Cate (*9*) on the decreasing resistance of

the pear tissue to pressure as the season advances. The pectose material is generally thought to be largely responsible for the thickening and cementing together of the cell walls and hence for the firm texture of fruit. The association of the decrease in amount of this and related material with decreasing resistance of the tissue to pressure is evidence in support of this theory.

The temperature at which the fruit was stored has no marked influence on this material. A comparison of the lots picked at the same time and stored under the different temperature conditions (Tables I–IV, columns 16–18) shows little variation.

INFLUENCE OF TIME OF PICKING AND TEMPERATURE OF STORAGE UPON PERCENTAGE OF DRY WEIGHT

In this report, sugars and acids have been figured to percentage of the wet weight, as it is considered that wet weight rather than dry weight percentage will give the most accurate index of quality. The data for total dry matter in the fruit are of much importance, however, especially in connection with the pear dehydration industry, and for the purpose of throwing light upon the question of how much shrinkage, due to loss of moisture, occurs in the fruit during storage.

From the data on dry weights presented in Tables I to IV it will be noted that while considerable variation seems to occur in various individual lots, one or two things stand out as of special interest. In the California fruit, in which the first pickings were made much in advance of the commercial season and when the fruit was very immature, the percentage of dry weight was higher in the earliest lots than it was in those lots picked during the main commercial shipping season, a month later. (Tables I and II, columns 19–22.) Toward the end of the season, however, the percentage of dry matter increased until the last pickings gave the highest dry-weight figures of all lots. The first pickings of the Oregon and Washington fruit (Tables III and IV) were made at a somewhat later relative date than the first pickings from California, so the fact that the earliest pickings show a low dry weight is in accord with the data for California sections.

Thus it is at once apparent that, for purposes of dehydration, pears left on the tree as long as possible will give not only the greatest tonnage, because of the size of the fruit, but will also give the greatest weight of the dried product per pound of green weight. Consequently, it is of special importance that pears intended for drying be left on the trees as long as possible.

If the dry weight of the fruit at the time of picking is compared with the dry weight of the same lots when they come from storage fully ripe it is seen that for well-matured fruit there is very little moisture loss during storage. A comparison of the earliest lots from Suisun and from Sacramento (Tables I and II) is interesting in that the Sacramento fruit was wrapped, whereas that from Suisun was loose in the box and unwrapped. There is no increase in dry weight in the Sacramento fruit,

while the early lots from Suisun show a marked increase during storage. This indicates the value of wrapping in preventing loss of moisture from fruit.

Examination of somewhat later lots picked during the commercial season shows no increase in dry weight while the fruit is in storage, and in many cases it shows an actual decrease. All the storages used were comparatively high in humidity, otherwise there might have been a loss due to more rapid evaporation from the fruit.

An examination of the lenticels of the fruit of the different lots was made as the fruit was freshly picked. A number of pears were put in methylene blue solution and after soaking a short time were removed and the lenticels examined under a microscope. It was found that the methylene blue readily penetrated the lenticels of the immature, early picked fruit. Fruit picked at the opening of the commercial season, however, had a layer of brown, suberized tissue formed in the lenticel, which prevented the penetration of the blue solution. Later in the season pears immersed for a considerable length of time and then rinsed in water showed only a very faint blue ring about the outside of the lenticel. The corky layer had apparently almost completely stopped penetration of the solution. Even when an immersed pear was placed under reduced pressure for a time and then under full atmospheric pressure the solution did not penetrate the lenticels.

With practice, this condition of the lenticels can be detected by the brown color of the corky growth without the use of a microscope and dye solution. It appears that this change in the lenticels may be a valuable aid to present methods of determining when the fruit is in condition to pick and handle without danger of shriveling or wilting.

EFFECT OF TIME OF PICKING UPON LENGTH OF TIME FRUIT MAY BE STORED

Table V shows the number of days between the time the fruit was picked from the tree and the time of full yellow ripeness. The Yakima fruit is not included, since the number of days in transit and the fact that one lot was delayed en route makes an accurate comparison impossible.

TABLE V.—*Number of days required for fruit to become soft, yellow ripe at different temperatures of storage*

Sacramento, Calif.			Suisun, Calif.			Medford, Oreg.		
Date of picking.	Number of days at 70° F.	Number of days at 40° F.	Date of picking.	Number of days at 70° F.	Number of days at 40° F.	Date of picking.	Number of days at 70° F.	Number of days at 40° F.
June 12	14	48	June 10	15	49	July 19	13	31
18	14	45	July 1	15	41	Aug. 8	13	26
July 5	14	32	10	14	......	28	11	23
12	14	33	22	15	32	......	......	......
Aug. 13	12	24	Aug. 6	12	28	......	......	......

The variations in the length of time required for the fruit from the different localities to become ripe in 40° F. storage may be due in part to the different lengths of time spent in transit to place of storage.

From this it is apparent that the results attained are similar to those found by other investigators—namely, that at the higher temperatures of storage, early picking gave somewhat longer keeping time than later picking. It has been impossible in this work to determine the relative keeping time at temperatures lower than 40° F. because of the necessity of removing the fruit from storage before it reached a full ripe condition.

At the 40° F. storage it was found, however, that the early fruits tended to scald and become brown rather than to ripen in good condition, while the later pickings ripened to full yellow and prime condition with practically no scald. Another very important observation was that although late-picked fruit tends to become yellow more quickly than early picked lots, it remains in firm, prime eating condition for a much longer period after becoming yellow than the fruit picked early.

GENERAL DISCUSSION OF RESULTS AS APPLIED TO COMMERCIAL HANDLING

The disposition of the commercial pear crop of the Pacific coast may be grouped under three divisions, which include practically the entire output—namely, (1) fresh shipment, for consumption as fresh fruit or for home canning; (2) commercial canning; and (3) drying or dehydration. The method of handling must, of necessity, be varied considerably, depending upon which of these methods of marketing is to be followed.

When pears are to be shipped fresh, certain factors other than those which determine the very highest quality of fruit must be considered. Fruit picked comparatively early in the season will remain sound somewhat longer, even at the lowest temperatures that it is possible to secure while the fruit is in transit, than will that picked too late; and this must always be an important consideration in determining the time to pick for fresh shipment. It must be remembered, however, that late-picked fruit is richer in sugar and of much higher dessert quality than fruit picked and shipped very early. Furthermore, while late-picked fruit, especially in the relatively high temperatures necessary in cars in transit, comes to prime eating condition in a shorter length of time, it remains in prime condition for a longer period, a consideration of much importance to the retail trade.

In the cannery and dehydrated fruit trades, it is possible to sacrifice something in keeping quality for a higher dessert quality product. Most of the fruit is utilized near the point of production. In the cannery industry the largest problem is to secure a good product and at the

same time to plan so that the tremendous tonnage that comes on within a short period is utilized before the fruit becomes overripe and breaks down. Almost every year canners lose a considerable quantity of pears because the fruit becomes overripe before the cannery can handle it.

The first consideration of the canner should be the securing of a high quality product by leaving the fruit on the trees until well developed. Pears picked very early are low in the natural fruit sugars and are of very inferior quality, whether eaten fresh or canned. A high-grade canned product can be secured only by using a high quality of fruit.

If this is done, it becomes practically necessary for the cannery man to store part of his season's supply. If certain conditions of storage are carried out, the keeping of Bartlett pears in storage, even up to two months, and still securing a high-quality product is a practical certainty. These conditions may be summarized briefly as follows:

(1) Use only well-developed fruit for storage. Early pickings tend to "scald" or turn brown and decay and break down much faster when removed from storage.

(2) Put fruit into storage immediately after it is picked. The maximum time that should elapse between picking and storing should not be more than three or four days. The cannery man will know the capacity of his plant; and, if more tons are being picked each day than he can handle, unless some go directly into storage, he can be sure that his cannery will be "flooded" when the fruit ripens. The fruit should go to the storage as soon as picked, rather than when it begins to soften. Much loss in pears in cold storage occurs because the fruit is in an almost soft-ripe condition when put in.

(3) Fruit should be cooled as quickly as possible after being placed in storage. It is especially desirable that a room with a large amount of direct expansion or brine piping be used, so that the temperature can be reduced quickly to 30° F. The fruit will cool somewhat more slowly than the air, although, if the fruit is loose in lug boxes, it will follow the air temperature rather closely.

(4) An even temperature should be maintained. If the storage rooms are large, it will be well worth while to use certain rooms for cooling down the fruit when it arrives, after which it may be transferred to other rooms for holding. This eliminates putting warm fruit into a room in which other fruit, already cooled, is being held. While this necessitates an extra handling, it is well worth while if it is desired to hold the fruit for some time. Especially is this system desirable if certain rooms having greater cooling capacity can be utilized for this precooling.

(5) The temperature should be held down to 28° or 30° F. if a long storage period is desired. Well-developed Bartlett pears will store at that temperature, ripen in excellent condition if removed at any time up to two or three months, and give a high-quality product. If it is desired

to hold the fruit for only a few weeks, somewhat higher temperatures are permissible; but even for short storage periods a low temperature, followed by the removal of the fruit and ripening at outside air temperature, gives a better product.

(6) The cooling capacity of the storage plant should not be overtaxed. It is possible in the case of Bartlett pears to "store on the tree" to a very marked extent. Two weeks' time on the tree makes only a small difference in the length of time pears will remain sound after removing from the tree, so for cannery trade it is not necessary to pick the entire crop within a very short time. Of course, other factors, such as amount of drop, load on the trees, etc., must be considered.

The foregoing suggestions presuppose a very close working agreement between producer, canner, and cold storage; and this is essential for successful handling of Bartlett pears through cold storage. The fruit must be sent to the storage plant quickly if it is to be held in storage, and the cooling capacity must be such that the fruit can be cooled down within a short time. The temperature and storage recommendations apply only to Bartlett pears, since other varieties have been found to give different responses under storage treatment (*8*, *9*).

For the dehydration of Bartlett pears, if a drying plant is used, the same principles apply as for canning. On the other hand, if sun drying is used, the problem is much simplified, as the fruit can be handled in almost any quantity within a short time. For drying, however, it is of twofold importance that the fruit remain on the trees as long as possible, for the quality is not only improved but the accumulation of sugars gives an increase in the weight of dried product per pound of green fruit.

SUMMARY

There is a marked and quite uniform increase in total sugar in Bartlett pears from early summer until after the time of the close of the commercial picking season. The increase during the latter part of the season is mainly due to an accumulation of sucrose, while the earlier increase is due mainly to reducing sugar.

A distinct relationship was found between the total amount of sugar present in the ripe fruit and the temperature of the storage at which it had been held from the time of removing from the tree until ripe. Pears ripened at 70° F. contained the highest percentage of sugar, those ripened at 40° possessed the lowest total sugar content, and those held at 30° for from 6 to 14 weeks and then ripened at room temperature were intermediate in amount of total sugar. There was no marked relation between temperature of storage and relative amount of sucrose and reducing sugar.

Percentage of titratable acid in the fruit tended to decrease in fruit from the California sections as the season advanced, while it tended to

increase in that from Oregon and Washington. There was an increase in acid between the time of picking and the time of full ripening of the fruit when held at 70° F. There was much less acid in fruit ripened at 40° than in that ripened at 70°, and still less in fruit that had been held at 30°. The acid content of the fruit that was allowed to become well matured on the tree remained nearly constant during storage.

There was a progressive reduction in the alcohol-insoluble, acid-hydrolyzable reducing material as the season advanced, not only in the fruit fresh picked from the tree, but also in the same fruit after ripening. There is a marked reduction in these substances between the time when the fruit is first picked and the time when the same fruit becomes ripe.

The percentage of total solids is lowest at about the opening of the commercial season. This tends to increase with the accumulation of sugar in the late-picked lots.

With proper precautions of picking, handling, and storing, Bartlett pears can be held two to three months in storage and then taken out in good condition.

LITERATURE CITED

(1) CLARK, W. BLAIR.
1917. A SAMPLING PRESS. *In* Jour. Indus. and Engin. Chem., v. 9, no. 8, p. 788-790.

(2) CRUESS, W. V., and STONE, P. M.
1916. PRELIMINARY OBSERVATIONS ON THE RIPENING OF BARTLETT PEARS. *In* Mo. Bul. State Com. Hort. [Calif.], v. 5, no. 12, p. 425-429.

(3) DUNBAR, P. B., and BIGELOW, W. D.
1913. THE ACID CONTENT OF FRUITS. (Abstract.) *In* Science, n. s. v. 38, no. 983, p. 639-640.

(4) EWERT.
1910. DIE KORRELATIVEN EINFLÜSSE DES KERNS BEIM REIFEPROZESS DER FRÜCHTE. *In* Landw. Jahrb., Bd. 39, Heft 3, p. 471-486, pl. 12-13.

(5) GORE, H. C.
1911. STUDIES ON FRUIT RESPIRATION. U. S. Dept. Agr. Bur. Chem. Bul. 142, 40 p., 17 fig.

(6) KELHOFER.
1899. WEITERE UNTERSUCHUNGEN ÜBER DIE VERTEILUNG VON ZUCKER, SÄURE UND GERBSTOFF IN DEN BIRNENFRÜCHTEN. *In* VI. & VII. Jahresb. Deut. Schweiz. Vers. Sta. u. Schule Obst-, Wein- u. Gartenbau Wädensweil, 1895/96-1896/97, p. 68-71.

(7) KULISCH, P.
1892. BEITRÄGE ZUR KENNTNISS DER CHEMISCHEN ZUSAMMENSETZUNG DER AEPFEL UND BIRNEN MIT BESONDERER BERÜCKSICHTIGUNG IHRER VERWENDUNG ZUR OBSTWEINBEREITUNG. *In* Landw. Jahrb., Bd. 21, p. 427-444.

(8) LEWIS, C. I., MAGNESS, J. R., and CATE, C. C.
1918. PRELIMINARY REPORT OF PEAR HARVESTING INVESTIGATIONS IN ROGUE RIVER VALLEY. Oreg. Agr. Exp. Sta. Bul. 154, 24 p.

(9) ——— MURNEEK, A. E., and CATE, C. C.
1919. PEAR HARVESTING AND STORAGE INVESTIGATIONS IN ROGUE RIVER VALLEY. (Second report.) Oreg. Agr. Exp. Sta. Bul. 162, 39 p., 10 fig.

(10) POWELL, G. Harold, and FULTON, S. H.
1903. COLD STORAGE, WITH SPECIAL REFERENCE TO THE PEAR AND PEACH. U. S. Dept. Agr. Bur. Plant Indus. Bul. 40, 26 p., 7 pl. (partly col.).

(11) RITTER, George.
1910. UEBER DEN CHEMISCHEN REIFUNGSPROZESS DER FRÜCHTE, MIT BESONDERER BERÜCKSICHTIGUNG DES OBSTES. *In* Deut. Obstbauztg., Jahrg. 1910, Heft 31, p. 429–435.

(12) RIVIÈRE, Gustave, and BAILHACHE, Gabriel.
1908. ÉTUDE RELATIVE A LA PROGRESSION ASCENDANTE DU SUCRE ET A LA PROGRESSION DESCENDANTE DE L'ACIDITÉ, DANS LES FRUITS POIRIER, DEPUIS LEUR FORMATION JUSQU'A LEUR MATURITÉ. *In* Jour. Soc. Nat. Hort. France, s. 4, t. 9, p. 284–289.

(13) ——— ———
1910. DE L'INFLUENCE DES FEUILLES QUI ACCOMPAGNENT IMMÉDIATEMENT LES FRUITS DU POIRIER, SUR LEUR ACCROISSEMENT EN POIDS ET SUR LEUR COMPOSITION CHIMIQUE. *In* Jour. Soc. Nat. Hort. France, s. 4, t. 11, p. 678–680.

(14) SHAMEL, A. D.
1917. SOME OBSERVATIONS UPON THE RELATION OF HUMIDITY TO THE RIPENING AND STORAGE OF FRUITS. *In* Mo. Bul. State Com. Hort. [Calif.], v. 6, no. 2, p. 39–41.

(15) STUBENRAUCH, A. V., and RAMSEY, H. J.
1913. BARTLETT PEAR PRECOOLING AND STORAGE INVESTIGATIONS IN THE ROGUE RIVER VALLEY. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 114, p. 19–24.

(16) TAYLOR, R. H., and OVERHOLZER, E. L.
1919. SOME EFFECTS OF HIGH TEMPERATURE AND HUMIDITY UPON THE KEEPING QUALITY OF BARTLETT PEARS. *In* Mo. Bul. State Com. Hort. [Calif.], v. 8, no. 3, p. 118–125.

(17) THOMPSON, Firman, and WHITTIER, A. C.
1913. FORMS OF SUGAR FOUND IN COMMON FRUITS. *In* Proc. Soc. Hort. Science, 9th Ann. Meeting, 1912, p. 16–22.

FURTHER DATA ON THE ORANGE-RUSTS OF RUBUS

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In 1916 the writer showed that there exists in the United States two orange-rusts on species of Rubus (*1*).[2] Morphologically these rusts closely resemble each other in their caeoma stages, but in the behavior of the orange spores when germinated and in life cycle they were shown to differ. During the last two seasons further observations were made on the orange-rusts, and it is the object of the present paper to report the results obtained in this study.

Atkinson (*2*) has performed some experiments which to him seemed to indicate that there is only one orange-rust on species of Rubus in the United States. He admits that the orange spores show two distinct methods of germination but attributes this to the influence of temperature. According to his view, promycelia are produced at high temperatures and germ tubes at low temperatures. He suggests that this may explain the behavior of the orange-rusts in different parts of the country. In the north and in mountainous regions where the spring temperatures are relatively low the aeciospores produce germ tubes, while in southern sections of the country where temperatures are high they produce promycelia.

The writer (*7*) has previously reported the behavior of the aeciospores of the two orange-rusts when germinated side by side at a room temperature of about 25° C. This experiment seemed to show conclusively that the aeciospores of the two rusts differ in manner of germination. Nevertheless, in view of Atkinson's results some further germination tests have been made.

GYMNOCONIA INTERSTITIALIS ON BLACK RASPBERRY

In the fall of 1916 the writer collected the telia of *Gymnoconia interstitialis* on leaves of wild black raspberry plants growing on the Virginia side of the Potomac River near Washington, D. C. These telia showed that the long-cycled rust is present in the locality just mentioned. Since that time many collections of orange-rust have been made from both wild and cultivated Rubus plants in the vicinity of the city of Washington. A study of these specimens has shown that the rust on the black raspberry is always long-cycled while the rust on the blackberry and

[1] The writer wishes to acknowledge here the help he has received from many colleagues who have offered suggestions or have sent him living specimens of the rusts.

[2] Reference is made by number (italic) to "Literature cited," p. 512.

dewberry plants, so far as has been observed, is short-cycled in this region. Wild blackberry and black raspberry plants occur abundantly along the Potomac River in both Maryland and Virginia. They are frequently intermingled with each other, and often both are infected with orange-rust. During the springs of 1917 and 1918 the two rusts were many times found growing close to each other, and during both seasons the telia of Gymnoconia were found occurring sparingly on leaves of wild black raspberry plants. The telia were always found on or near those plants that had borne caeomas of Gymnoconia. They were never found on any blackberry host. Many cultivated blackberry and black raspberry fields in the vicinity of Washington are troubled with orange-rust. In every instance the germination tests have shown that the raspberry plants are infected with the long-cycled rust. Rust found in the cultivated blackberry fields is always the short-cycled form.

Plate 92 shows the way the aeciospores of the two rusts germinate on Beyerinck agar at room temperature (about 25° C.). The spores shown in Plate 92, A, were taken from leaves of wild black raspberry at West Falls Church, Va. They have produced long germ tubes. Those shown in Plate 92, B, were collected at the same place on wild blackberry. They have produced promycelia-bearing sporidia.

INFLUENCE OF TEMPERATURE ON GERMINATION

In order to study the effect of temperature on germination numerous collections were made from both wild and cultivated blackberry and raspberry plants. The spores were germinated in Petri dishes on water and on Beyerinck agar. The cultures were incubated at temperatures varying by 5° intervals and ranging from 0° to 30° C. None of the spores of either rust germinated at 0°. At 5° excellent germination was obtained, but growth was slow. At all of the higher temperatures—10°, 15°, 20°, 25°, and 30°—the spores of the two rusts germinated equally well. It was noted that at low temperatures such as 5° and 10° the spores of the long-cycled rust began to germinate somewhat sooner than those of the short-cycled rust. Germination in cultures of both kinds of spores took place more rapidly at 30° than at any of the lower temperatures. Fewer spores germinated, however, at this temperature than at the lower temperatures. The spores of both rusts germinated well at all the temperatures tested between 0° and 30°.

Spores taken from blackberry leaves always produced promycelia, while those from the black raspberry leaves produced long germ tubes. Mature aeciospores of the two rusts collected at the same time and often within a few feet of each other and incubated at the same temperatures and on the same media always showed the same differences in manner of germination. The promycelia produced by the spores from blackberry leaves are typical in every way. They become divided into

four or more cells, and usually four of these contain one nucleus each. Each nucleated cell is capable of producing a sporidium. The germ tubes arising from spores borne on raspberry leaves are long and sinuous. By suitable methods of staining they have been shown to contain two nuclei. At an early stage in germination they may be distinguished from promycelia by their smaller diameter and more rapid longitudinal growth. Temperature, within the range tested, has no effect on the manner in which the aeciospores of these two rusts germinate. In the vicinity of Washington, D. C., at Mountain Lake, Va., and at French Creek, W. Va., both rusts occur side by side under the same conditions of temperature and climate. The writer is, therefore, unable to accept the theory that temperature determines whether spores of a given orange-rust specimen will produce germ tubes or promycelia.

COLOR OF SPORES IN MASS

The finding of the two orange-rusts growing within a short distance of the laboratories of the Bureau of Plant Industry made it easy for the writer to compare them more carefully than was possible when they had to be brought from different parts of the country. The comparison of the rusts as they occur side by side on their living hosts has brought to light certain differences that were not noticed earlier. One of the most important of these is the color of the spores in mass.

It soon became evident that the spores of the short-cycled rust are lighter in color than those of Gymnoconia. The spore colors of the two rusts were matched on Ridgeway's color chart. According to this chart the spores of the short-cycled rust are cadmium orange, while those of the long-cycled rust are xanthine yellow. These two colors do not differ greatly from each other and stand side by side in the chart. Nevertheless they can be easily distinguished after one has once noted the difference between them. It is surprising that this difference was not seen earlier, especially since account was taken of the color of the spores in mass. It seems that failure in this regard was due to the fact that the color of the spores of both rusts begins to fade within a few weeks after they are collected, and differences in shade of color were attributed to fading.

It was at first thought that the difference in color between the spores on raspberry and on blackberry leaves might be due to the difference in host. In order to test this hypothesis a number of collections were made during the spring of 1917 and 1918. The long-cycled rust was collected on both wild and cultivated black raspberry at French Creek, W. Va. It was collected on wild blackberry (identified as *Rubus alleghaniensis*) at Mountain Lake, Va. Numerous collections were made in the Adirondack Mountains near Old Forge, N. Y., and in the White Mountains near Glen and Jackson, N. H. It was also collected

on black raspberry at Rouses Point, N. Y. Collections of the short-cycled rust were made on wild dewberry and wild blackberry at French Creek, W. Va., on wild dewberry at Mountain Lake, Va., and on wild blackberry and dewberry plants at many other points.

The material collected in 1917 and 1918 was brought together for comparison at the end of each season and before there was serious fading in the color of the spores. This comparison has shown that for the material at hand the two orange-rusts exhibit the same color differences regardless of the hosts on which they occur or the localities from which they are collected. The color difference makes it possible to identify the two rusts in the field without resort to spore germination.

Plate D illustrates the difference in the color of the spores in mass. Figure 1 shows an infected black raspberry leaf, figure 2 an infected blackberry leaf.

MORPHOLOGY OF AECIOSPORES

In a former paper the writer (7) has pointed out that no morphological differences could be observed between the aeciospores of certain specimens of the two orange-rusts. While this statement was true for the specimens under study, it does not hold when larger numbers of specimens of the two rusts are compared. A study of more than 100 different collections has shown that the spores of the two rusts differ considerably from each other both in size and in shape. While a few specimens may not reveal this fact, a more extended study shows that the aeciospores of the two rusts are, on the whole, morphologically different.

In order to show this difference more clearly than is possible by description, an outline drawing has been made of a few typical aeciospores from a number of different specimens of the two rusts. The drawings were made with the aid of a camera lucida. The same magnification was used for all spores, so that the different drawings may be readily compared. There is always a certain amount of variation in the size and shape of the spores of a given specimen. This is greater for some specimens than for others, and it was not always easy to select spores that would be typical. Before material for drawing was chosen, spores from several mature caeomas on each specimen were transferred to separate drops of water on glass slides. They were then observed under the microscope, and a group was finally chosen that seemed to be typical for the specimen in question. Table I gives information regarding the place and time of collection, host, manner of germination, and color of spores in mass for most of the specimens collected in 1917 and 1918. Numbers given in the last column of the table indicate the drawings in Plates 93 and 94, which show an average sample of spores for each specimen.

TABLE I.—*Place and time of collection, host, manner of germination, and color of the aeciospores in mass for most of the specimens collected in 1917 and 1918*

Place of collection.	Time of collection.	Host.	Manner of germination.	Color of spores in mass.	Plate No.
Falmouth, Mass	June 20, 1918	Wild dewberry	Promycelia	Cadmium orange	93, fig. 1
Arlington, Va	June, 1918	Wild blackberry	do	do	93, fig. 2
Massachusetts	June 27, 1917	do	do	do	93, fig. 3
Berlin, Md	June 8, 1917	Cultivated blackberry	do	do	93, fig. 4
Hyattsville, Md	June 15, 1918	Wild dewberry	do	do	93, fig. 5
West Falls Church, Va.	May 21, 1918	do	do	do	93, fig. 6
Auburn, Ala	Apr. 27, 1918	do	do	do	93, fig. 7
Fayetteville, Ark	June 7, 1917	Wild blackberry	do	do	93, fig. 8
Potomac Heights, D. C.	June, 1917	do	do	do	93, fig. 9
Morgantown, W. Va.	June 7, 1918	Cultivated blackberry, variety Eldorado.	do	do	93, fig. 10
Blacksburg, Va	June 11, 1918	Cultivated blackberry, variety Iceberg.	do	do	93, fig. 11
Ithaca, N. Y	June 4, 1918	Wild blackberry	do	do	93, fig. 12
Blacksburg, Va	May 25, 1917	do	do	do	93, fig. 13
West Falls Church, Va.	May 21, 1918	do	do	do	93, fig. 14
Gainesville, Fla	Mar. 25, 1918	*Rubus cuneifolius*	do	do	93, fig. 15
Ithaca, N. Y	June, 1918	Wild blackberry	do	do	93, fig. 16
Chico, Calif	May 1, 1918	*R. ursinus*	do	do	93, fig. 17
Ithaca, N. Y	June 4, 1918	Wild dewberry	do	do	93, fig. 18
Arlington, Va	June, 1918	Wild blackberry	do	do	93, fig. 19
Berkeley, Calif	Mar. 30, 1918	*R. parviflorus*	do	do	93, fig. 20
Hammonton, N. J	June 20, 1918	Cultivated blackberry	do	do	93, fig. 21
Fayetteville, Ark	June 7, 1917	Wild blackberry	do	do	93, fig. 22
West Falls Church, Va.	May 21, 1918	do	do	do	93, fig. 23
French Creek, W. Va.	June 8, 1918	Wild dewberry	do	do	93, fig. 24
Congress Heights, D. C.	May 20, 1918	Wild blackberry	do	do	93, fig. 25
Connellsville, Pa	June 6, 1918	do	do	do	93, fig. 26
Chico, Calif	May 19, 1918	*R. ursinus*	do	do	93, fig. 27
Vienna, Va	May 21, 1918	Wild blackberry	do	do	93, fig. 28
Bryan, Ohio	June 12, 1918	Cultivated blackberry.	Germination very poor.	Color faded	93, fig. 29
Cameron, N. C	June 4, 1918	Wild blackberry	Promycelia	Cadmium orange.	93, fig. 30
Athens, Ohio	June 11, 1918	do	do	do	93, fig. 31
Blacksburg, Va	do	Cultivated blackberry, variety Early King.	do	do	93, fig. 32
Chico, Calif	May 1, 1918	*R. ursinus*	do	do	93, fig. 33
Arlington, Va	June, 1918	Wild blackberry	do	do	93, fig. 34
Ithaca, N. Y	do	Wild dewberry	do	do	93, fig. 35
Mountain Lake, Va	June 11, 1918	*R. americanus*	do	do	93, fig. 36
French Creek, W. Va.	June 8, 1918	Wild dewberry	do	do	93, fig. 37
Blacksburg, Va	June 11, 1918	Cultivated blackberry	do	do	93, fig. 38
Do	do	Cultivated blackberry, variety Mersereau.	do	do	93, fig. 39
Vienna, Va	do	Cultivated blackberry	do	do	93, fig. 40
Ithaca, N. Y	June 4, 1918	Wild blackberry	do	do	93, fig. 41
Blacksburg, Va	June 11, 1918	Cultivated blackberry, variety Ancient Britain.	do	do	93, fig. 42
Janassee Junction, Ga.	May 14, 1918	Wild blackberry	do	do	93, fig. 43
Thunderbolt, Ga	Mar. 17, 1918	*R. procumbens*	do	do	93, fig. 44
Hyattsville, Md	June 15, 1918	Wild dewberry	do	do	93, fig. 45
Do	do	Wild blackberry	do	do	93, fig. 46
Blacksburg, Va	June 11, 1918	Cultivated blackberry.	do	do	93, fig. 47
Vienna, Va	May 29, 1918	Wild blackberry	do	do	93, fig. 48
Willard, N. C	June 26, 1917	do	do	do	93, fig. 49
Connellsville, Pa	June 8, 1918	do	do	do	93, fig. 50
Auburn, Ala	Mar. 4, 1918	do	do	do	93, fig. 51
Butte Creek Cányon, Calif.	May 19, 1918	*R. ursinus*	do	do	93, fig. 52
Stark, Fla	1917	Wild blackberry	do	do	93, fig. 53
Blacksburg, Va	June 11, 1918	do	do	do	93, fig. 54
Thunderbolt, Ga	Mar. 17, 1918	*R. hispidus*	do	do	93, fig. 55
Hyattsville, Md	June 15, 1918	Wild blackberry	do	do	93, fig. 56
Blacksburg, Va	May 25, 1917	do	do	do	93, fig. 57
Hammond, La	Mar. 29, 1918	do	do	do	93, fig. 58
Orlando, W. Va	June 8, 1918	do	do	do	93, fig. 59
Ithaca, N. Y	June 4, 1918	do	do	do	93, fig. 60
Blacksburg, Va	June 12, 1918	do	do	do	93, fig. 61
West Falls Church, Va.	May 21, 1918	Cultivated blackberry	do	do	93, fig. 62
Arlington, Va	June, 1918	Wild blackberry	do	do	93, fig. 63
Do	do	do	do	do	93, fig. 64
French Creek, W. Va.	June 8, 1918	do	do	do	93, fig. 65
Madrid, Me	July 3, 1917	do	Germ tubes	Xanthine yellow	94, fig. 1
French Creek, W. Va.	June 8, 1918	Black raspberry	do	do	94, fig. 2
Glen, N. H	June 22, 1917	*R. nigrobaccus*	do	do	94, fig. 3

TABLE I.—*Place and time of collection, host, manner of germination, and color of the aeciospores in mass for most of the specimens collected in 1917 and 1918*—Continued

Place of collection.	Time of collection.	Host.	Manner of germination.	Color of spores in mass.	Plate No.
West Falls Church, Va.	May 21, 1918	Cultivated raspberry	Germ tubes	Xanthine yellow	94, fig. 4
Vienna, Va	do	do	do	do	94, fig. 5
Old Forge, N. Y	June 27, 1918	*R. canadensis*	do	do	94, fig. 6
Glen, N. H	June 22, 1918	Wild blackberry	do	do	94, fig. 7
Mountain Lake, Va.	June 11, 1918	*R. alleghainensis*	do	do	94, fig. 8
West Falls Church, Va.	May 21, 1918	Cultivated black raspberry.	do	do	94, fig. 9
Portland, Me	June 24, 1917	*R. canadensis*	do	do	94, fig. 10
Madison, Wis	June 4, 1918	Wild blackberry	do	do	94, fig. 11
Phillips, Me	July 3, 1917	do	do	do	94, fig. 12
Portland, Me	June 24, 1917	*R. canadensis*	do	do	94, fig. 13
East Lansing, Mich	June 30, 1917	Wild blackberry	do	do	94, fig. 14
Sebago Lake, Me	June 23, 1917	*R. triflorus*	do	do	94, fig. 15
Michigan	June, 1917	Wild blackberry	do	do	94, fig. 16
Old Forge, N. Y	June 26, 1918	*R. canadensis*	do	do	94, fig. 17
Madison, Wis	July 17, 1917	Wild blackberry	do	do	94, fig. 18
Old Forge, N. Y	June 27, 1918	*R. canadensis*	do	do	94, fig. 19
West Falls Church, Va.	May 21, 1918	Wild black raspberry	do	do	94, fig. 20
Do	July 4, 1917	Cultivated black raspberry.	do	do	94, fig. 21
Do	May 21, 1918	do	do	do	94, fig. 22
Glen, N. H	June 22, 1917	*R. canadensis*	do	do	94, fig. 23
Mountain Lake, Va.	June 11, 1918	*R. alleghaniensis*	do	do	94, fig. 24
French Creek, W. Va.	June 8, 1918	Black raspberry	do	do	94, fig. 25
Portland, Me	June 24, 1917	*R. canadensis*	do	do	94, fig. 26
Smugglers Notch, Vt.	July 11, 1917	*R. strigosus*	do	do	94, fig. 27
Madrid, Me	July 3, 1917	Wild blackberry	do	do	94, fig. 28
Old Forge, N. Y	June 27, 1918	*R. canadensis*	do	do	94, fig. 29
Smugglers Notch, Vt.	July 11, 1917	do	do	do	94, fig. 30
Bancroft, Wis	July, 1917	*R. hispidus*	do	do	94, fig. 31
Julet, N. Y	June 26, 1918	*R. canadensis*	do	do	94, fig. 32
French Creek, W. Va.	June 6, 1917	Black raspberry	do	do	94, fig. 33
Old Forge, N. Y	June 26, 1918	*R. canadensis*	do	do	94, fig. 34
Madison, Wis	June 4, 1918	Wild blackberry	do	do	94, fig. 35
Portland, Me	June 24, 1917	*R. triflorus*	do	do	94, fig. 36
Sebago, Lake, Me	June 23, 1917	do	do	do	94, fig. 37
Smugglers Notch, Vt.	July 11, 1917	*R. canadensis*	do	do	94, fig. 38
Sebago Lake, Me	June 23, 1917	*R. triflorus*	do	do	94, fig. 39
Rouses Point, N. Y	June 29, 1918	Black raspberry	do	do	94, fig. 40
Sebago Lake, Me	June 23, 1917	Wild blackberry	do	do	94, fig. 41
Mountain Lake, Va.	June 11, 1918	*R. alleghaniensis*	do	do	94, fig. 42
Bound Brook, N. J	June 17, 1918	Black raspberry	No germination.	do	94, fig. 43
Glen, N. H	June 22, 1916	*R. canadensis*	Germ tubes	Color faded	94, fig. 44

Plate 93 shows spores from 65 different specimens of the short-cycled orange-rust. Figures 1 to 44 on Plate 94 show spores from 44 different specimens of the long-cycled rust. The figures on these plates demonstrate that, on the whole, the aeciospores of the two rusts are morphologically different. The spores of the short-cycled rust are smaller than those of the long-cycled. They are also more angular and more elongated. Their shape is more irregular. It will be seen that the size and shape of the aeciospores from different specimens of the two rusts vary considerably. On this account it is not always possible by observing spores under the microscope to determine with certainty to which group a given specimen may belong. On the other hand, spore characters do make possible a rather accurate separation of specimens belonging to the two rusts. It is difficult to say just how accurate such determinations will be. Much depends on the specimens at hand and on the judgment of the one who undertakes such a task. The writer's determinations by this means have proved to be correct in about 85

per cent of the specimens studied. By this method it is possible to identify with a fair degree of accuracy orange-rust specimens in herbaria long after the spores are dead and have lost their color. It must be remembered, however, that it is always necessary to have a liberal quantity of mature spores in order to make determinations of value.

The figures on Plate 93 show the variation in the size and shape of spores from different collections of the short-cycled rust. The spores shown in most of the figures are relatively small and angular. Those shown in figures 9, 13, 39, 45, and 55 are large and round. They look like the spores of the long-cycled rust, but their color and manner of germination prove that they belong to the short-cycled rust. Figures 3, 10, 28, 29, 33, 35, 36, 44, and 57 show spores that resemble somewhat those of the long-cycled rust. The specimens from which these aeciospores were taken can not be satisfactorily identified on the basis of spore characters. The spores shown in all of the other figures on Plate 93 are characteristic for the short-cycled rust; but even in such cases one can not be absolutely sure that they belong to this fungus, for occasionally a specimen of the long-cycled rust bears spores like those shown in figure 35 of Plate 94. The aeciospores shown in this figure are small and angular; they do not look like spores of the long-cycled rust. Spores shown in figures 4, 6, 8, 9, 19, 25, 42, and 43 of Plate 94 resemble to a certain degree spores of the short-cycled rust. On the whole, however, aeciospores from different samples of the long-cycled rust are more uniform as regards size and shape than are those of the short-cycled rust.

GENETIC RELATIONSHIP BETWEEN THE TWO ORANGE-RUSTS

Although the two orange-rusts differ from each other in several characters, it must not be denied that they are alike in many respects. They are both systemic on species of Rubus. Their caeomas look much alike, and in many specimens the aeciospores are quite similar. These points of resemblance suggest a genetic relationship. Along with the further evidence that the two rusts are distinct and different from each other have come certain facts that strengthen this suggestion. In an earlier paper the writer mentioned finding a promycelium in a culture of aeciospores of the long-cycled rust. It was thought at the time that the spore producing the promycelium might have entered as a contamination. During the spring of 1917 and 1918 aeciospores of Gymnoconia collected in different parts of the country were germinated in great numbers. Each culture was carefully examined under the microscope. In many cultures only germ tubes could be found. A few promycelial germinations have been obtained, however, from spores of every collection of Gymnoconia which the writer made during the last two seasons. Sometimes such germinations are exceedingly rare,

but if enough spores are germinated the promycelia will be found. The spores of some collections produce them more often than those of other collections. In general it may be said that promycelia are produced more abundantly by aeciospores collected late in the season. They are not entirely absent, however, from cultures made with spores collected early in the season. The possibility of these promycelia being produced by spores of the short-cycled rust that have contaminated the cultures has been excluded in most instances. In order to do this, the aeciospores were taken from caeomas that had not yet opened. Moreover, many of the spores were collected and germinated in parts of the country where the short-cycled rust is not known to occur. If the promycelia appeared only in cultures from aeciospores collected in the South where the short-cycled rust is abundant, mixed infection might offer a possible explanation. But since they also occur in cultures of spores collected in the North where the short-cycled rust has never been found, this explanation is unsatisfactory. In the vicinity of Glen, N. H., orange-rust has been collected each spring since 1913. Spores taken from a number of different places in this vicinity have been germinated, but the short-cycled rust has not been found. In both 1917 and 1918, cultures made at Glen were studied and found to contain a few promycelia. Promycelia have also been observed in cultures of the aeciospores of the long-cycled rust collected at Old Forge, N. Y., where several seasons' search has failed to reveal the presence of the short-cycled rust. They have been found in cultures of aeciospores taken from the black raspberry at Rouses Point, N. Y., French Creek, W. Va., and at points in the vicinity of Washington, D. C. Promycelia were also found in cultures of aeciospores collected at Mountain Lake, Va., on *Rubus alleghaniensis*.

When spores of the short-cycled rust are incubated at room temperature (about 25° C.) on a favorable medium, they produce promycelia-bearing sporidia within 24 hours. Spores of the long-cycled rust placed under similar conditions produce long germ tubes within 24 hours. Promycelia have seldom been found in these cultures after so short a time. They occur in cultures of Gymnoconia only after a rather long period of incubation. They can usually be found after 3 or 4 days. In order to study the production of promycelia by the aeciospores of Gymnoconia, it is best to incubate cultures at a fairly low temperature. Temperatures varying from 10° to 15° are favorable. Promycelia are always slow to make their appearance in cultures of this rust. If incubation temperatures are high, many germ tubes die before they have time to develop into promycelia. Moreover, cultures kept at high temperatures are usually overgrown by saprophytic mold fungi and bacteria after a few days. Low temperatures check the growth of these organisms. Promycelia can be found most easily in

cultures of aeciospores kept at about 10° for a week or longer. It must be understood, however, that they are not produced very abundantly even under favorable conditions. Sometimes 1,000 germinated spores may be observed without the finding of a single promycelium, but usually several promycelia will be found for each 1,000 spores observed if the cultures are more than 4 days old. In some cultures they occur more abundantly.

It is interesting to note that most of the promycelia developing in cultures of the aeciospores of the long-cycled rust are abnormal, though normal ones are also present. Many of the abnormal promycelia produce one or more normal sporidia, and there can be no doubt regarding their true nature. The abnormal promycelia and their tardy appearance in the cultures seem to the writer to suggest that nuclear fusions and the subsequent reduction divisions are steps accomplished with difficulty in these spores. It would be highly interesting to study these phenomena cytologically, but the relatively small number of promycelial germinations makes such a task rather difficult.

From a study of many abnormal promycelia the writer has come to recognize certain structures as indicating an attempt at the production of sporidia. Some of these are cross walls, branches, especially those having a diameter less than that of the germ tube, and sporidia-like processes borne on structures that show more or less resemblance to sterigmata. In cultures of the aeciospores of the long-cycled orange-rust it is possible to find all gradations between normal promycelia and germ tubes that can hardly be recognized as promycelia at all. In order to show some of the stages between these two extremes a few drawings have been made of abnormal promycelia.

Figure 53 of Plate 94 shows a tube with two rather typical sporidia borne on typical sterigmata. No cross walls occur in this tube. Figure 49 shows a branched germ tube. A cross wall occurs just below the branch. No sporidia are borne on this tube, but there can be little doubt that this is an attempt at promycelium production. Figure 51 shows a germ tube with one cross wall and forked branches. One of these branches has produced a rather long club-shaped tube, while the other has developed into a sterigma-like process bearing a typical sporidium. A tube with one cross wall and several branches of small diameter is shown in figure 50. One of these branches is pointed like a sterigma and bears a spore that resembles a sporidium. Figure 45 represents a tube having one cross wall and several short branches. One of these branches is considerably enlarged toward its distal end and presents curves that suggest those of the normal sporidium. A short tube is shown in figure 47. This tube has one short branch which bears a sporelike body having curves that closely resemble those of a sporidium. The curves of the upper end of this body are especially like those of the

upper part of normal sporidia. Another short tube is shown in figure 46. This bears a branch with an enlarged end resembling a sporidium. A constriction at the point of the first bend in the branch would give rise to a fairly normal sporidium. Figure 48 shows a similar branch, but this time it arises from a very long germ tube. An unbranched tube is shown in figure 54. The diameter of the distal end of this tube is much less than that of the average diameter of germ tubes. Such a decrease in diameter frequently accompanies cross-wall production, branching, and other indications of an attempt at the production of sporidia. Figure 52 shows a tube with a branch of small diameter and one cross wall. The end cell has broken away from the remainder of the tube.

It must not be supposed that abnormal promycelia are uncommon in cultures of the short-cycled orange-rust or in cultures of germinating teliospores in general. Abnormal promycelia much like those described above have occasionally been found in cultures of the short-cycled rust. They are not common, however, under ordinary conditions of germination. In cultures of the aeciospores of Gymnoconia, on the other hand, most of the promycelia produced are abnormal.

The production of promycelia by the aeciospores of *Gymnoconia interstitialis* seems to the writer to be strong evidence that a close genetic relationship exists between the two orange-rusts. One of them is a typical short-cycled rust. It produces three kinds of spores: Spermatia, aeciospores, and sporidia. So far as the writer has observed it possesses one and only one life cycle. There is nothing unusual about this rust. The other orange-rust is long-cycled, but it is not a typical long-cycled rust. It is unusual in that it possesses two life cycles. In addition to the long cycle there is a much repressed short cycle, as shown by the occasional production of promycelia. We know that the germ tubes produced by these spores reinfect Rubus leaves. It is not known whether the sporidia can cause infection. Some of the sporidia have been seen to germinate. They appear normal in every way, and there seems to be no reason why they should not function.

So far as the writer knows no one has yet observed the production of promycelia in cultures of the European orange-rust of Rubus. Both Fischer (4) and Lindfors (*8*) have recently studied the manner of germination of the spores of this rust and have observed only germ tubes. Fischer, however, has shown a branch of small diameter coming from the end of one of his germ tubes. This suggests an attempt at promycelium production and leads the writer to believe that if large numbers of aeciospores of the European orange-rust are germinated and carefully observed promycelia will be found.

The findings of an occasional promycelium in cultures of the aeciospores of *Gymnoconia interstitialis* at once raised the question as to whether or not such a performance is usual among the rusts. It is not possible to

get much information on the question from the literature on the germination of rust spores. Most workers have not germinated aeciospores in large numbers, and a few promycelia in their cultures might easily have been overlooked. In order to settle this point it would be necessary to germinate the aeciospores of many different rusts in large numbers, and the writer has not undertaken this task. Nevertheless, it has seemed desirable to make a thorough study of the aeciospore germination of some other rust. For this study the aeciospores of *Aecidium fraxini* were chosen. These aeciospores are produced in large numbers and germinate readily on both water and Beyerinck agar. *A. fraxini* was found in abundance on black ash trees growing along the shore of Lake Champlain near Rouses Point, N. Y. Many cultures were made with aeciospores of this rust. The germinations were carefully observed, but not a single promycelium was ever found. Long, wavy germ tubes were produced. No cross walls or branches were observed. These spores produce germ tubes only.

Promycelia in cultures of the aeciospores of *Gymnoconia interstitialis* indicate that the two nuclei which ordinarily pass out into the germ tube and remain apart through many nuclear and cell divisions occasionally fuse in the spore or perhaps in the young germ tube. If we assume that reduction in chromosome number occurs here as in other promycelia and that the sporidia produced are capable of reinfecting the host, then *G. interstitialis* has a double life cycle such as has not been demonstrated for any other rust.

It is not believed that promycelia are commonly produced even in small numbers by the aeciospores of most rusts. On the other hand, it seems probable that other rusts will be found that possess double life cycles. Eriksson (*3*) has reported that the aecia of *Aecidium graveolens* which occur on species of Berberis are able to reproduce themselves, although they may also infect *Avena elatior* and give rise to *Puccinia arrhenatheri*. This strange behavior, which has never been accounted for, may be due to the production of promycelia by a certain number of the aeciospores. Recently Klebahn (*5*) reports that the aecia of *Peridermium pini* reproduce themselves on the pine. He states that the aeciospores give germ tubes, but a further study may show that some of them produce promycelia.

In an earlier paper (*6*) the writer expressed the opinion that the short-cycled orange-rust is more primitive than the long-cycled one. The fact that the long-cycled rust has a double life cycle is further evidence in favor of this view.

Arthur (*1*) considers the differences between the two orange-rusts sufficient to place them in separate genera. Moreover, these genera are widely separated in his classification. It would seem that the evidence of a genetic relationship between these rusts should be given consideration in any natural system of classification.

LITERATURE CITED

(1) ARTHUR, J. C.

1917. ORANGE RUSTS OF RUBUS. *In* Bot. Gaz., v. 63, no. 6, p. 501–515, map.

(2) ATKINSON, George F.

1918. SELECTED CYCLES IN GYMNOCONIA PECKIANA. *In* Amer. Jour. Bot., v. 5, no. 2, p. 79–83.

(3) ERIKSSON, Jakob.

1898. STUDIEN ÜBER DEN HEXENBESENROST DER BERBERITZE (PUCCINIA ARRHENATHERI KLEB.) *In* Beitr. Biol. Pflanz., Bd. 8, p. 1–16, 3 pl. Literatur, p. 14–15.

(4) FISCHER, Ed.

1916. MYKOLOGISCHE BEITRÄGE. *In* Mitt. Naturf. Gesell. Bern, 1915, p. 214–234, 2 fig.

(5) KLEBAHN, H.

1905. KULTURVERSUCHE MIT ROSTPILZEN. XII. BERICHT (1903 UND 1904). *In* Ztschr. Pflanzenkrank., Bd. 15, Heft 2, p. 65–108, 4 fig., pl. 3.

(6) KUNKEL, L. O.

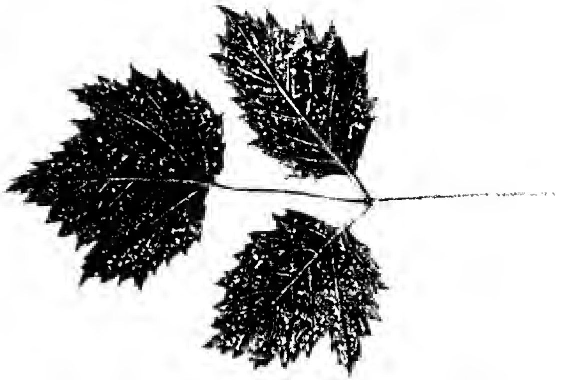
1914. NUCLEAR BEHAVIOR IN THE PROMYCELIA OF CAEOMA NITENS BURRILL AND PUCCINIA PECKIANA HOWE. *In* Amer. Jour. Bot., v. 1, no. 1, p. 37–47, pl. 3. Bibliography, p. 45–46.

(7) ———

1916. FURTHER STUDIES OF THE ORANGE RUSTS OF RUBUS IN THE UNITED STATES. *In* Bul. Torrey Bot. Club, v. 43, no. 10, p. 559–569, 5 fig.

(8) LINDFORS, Thore.

1918. MYKOLOGISCHE NOTIZEN. *In* Svensk Bot. Tidskr., bd. 12, no 2, p. 221–227, 4 fig.



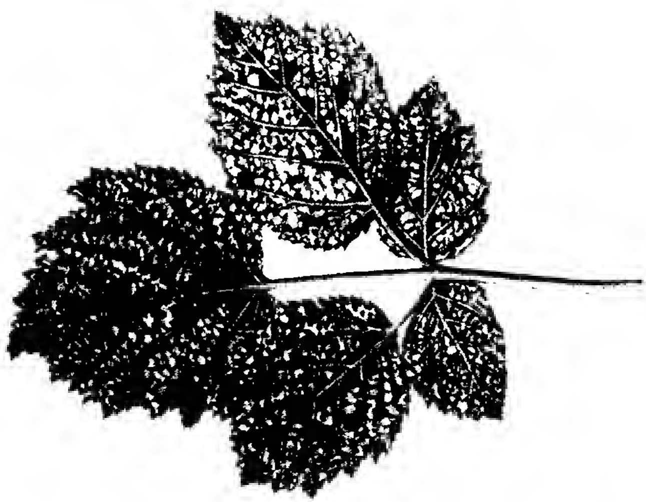


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PLATE D

1.—Infected black raspberry leaf covered with the caeomas of *Gymnoconia interstitialis*. The spores in mass are xanthine yellow.

2.—Blackberry leaf infected with the short-cycled orange-rust. The spores of this rust are cadmium orange in color.

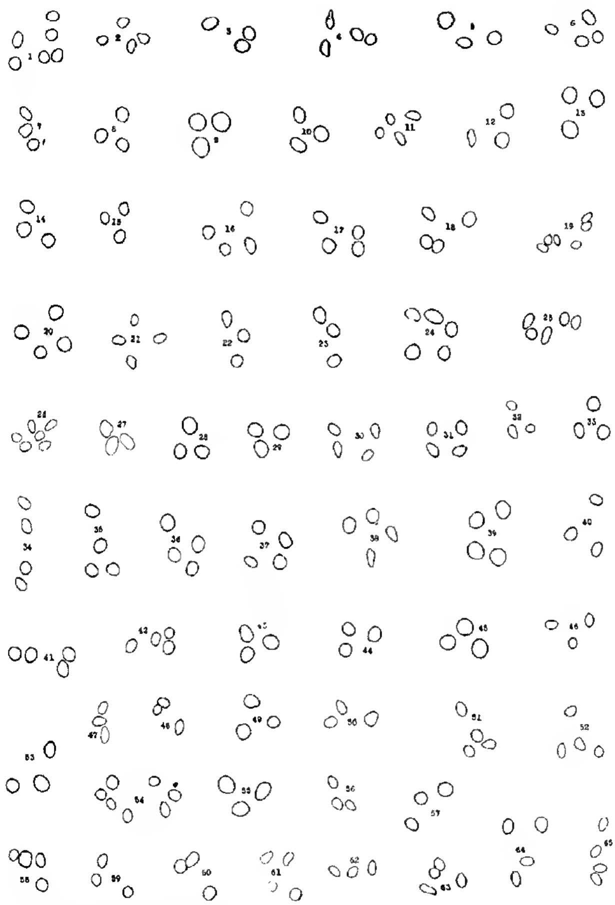

PLATE 93

Short-cycled orange-rust. ×100.

1.—Spores collected at Falmouth, Mass., on wild dewberry.
2.—Spores collected at Arlington, Va., on wild blackberry.
3.—Spores collected in Massachusetts on wild blackberry.
4.—Spores collected at Berlin, Md., on cultivated blackberry.
5.—Spores collected at Hyattsville, Md., on wild dewberry.
6.—Spores collected at West Falls Church, Va., on wild dewberry.
7.—Spores collected at Auburn, Ala., on wild dewberry.
8.—Spores collected at Fayetteville, Ark., on wild blackberry.
9.—Spores collected at Potomac Heights, D. C., on wild blackberry.
10.—Spores collected at Morgantown, W. Va., on cultivated blackberry, variety Eldorado.
11.—Spores collected at Blacksburg, Va., on cultivated blackberry, variety Iceberg.
12.—Spores collected at Ithaca, N. Y., on wild blackberry.
13.—Spores collected at Blacksburg, Va., on wild blackberry.
14.—Spores collected at West Falls Church, Va., on wild blackberry.
15.—Spores collected at Gainesville, Fla., on *Rubus cuneifolius.*
16.—Spores collected at Ithaca, N. Y., on wild blackberry.
17.—Spores collected at Chico, Calif., on *R. ursinus.*
18.—Spores collected at Ithaca, N. Y., on wild dewberry.
19.—Spores collected at Arlington, Va., on wild blackberry.
20.—Spores collected at Berkeley, Calif., on *R. parviflorus.*
21.—Spores collected at Hammonton, N. J., on cultivated blackberry.
22.—Spores collected at Fayetteville, Ark., on wild blackberry.
23.—Spores collected at West Falls Church, Va., on wild blackberry.
24.—Spores collected at French Creek, W. Va., on wild dewberry.
25.—Spores collected at Congress Heights, D. C., on wild blackberry.
26.—Spores collected at Connellsville, Pa., on wild blackberry.
27.—Spores collected at Chico, Calif., on *R. ursinus.*
28.—Spores collected at Vienna, Va., on wild blackberry.
29.—Spores collected at Bryan, Ohio, on cultivated blackberry.
30.—Spores collected at Cameron, N. C., on wild blackberry.
31.—Spores collected at Athens, Ohio, on wild blackberry.
32.—Spores collected at Blacksburg, Va., on cultivated blackberry, variety Early King.
33.—Spores collected at Chico, Calif., on *R. ursinus.*
34.—Spores collected at Arlington, Va., on wild blackberry.
35.—Spores collected at Ithaca, N. Y., on wild dewberry.
36.—Spores collected at Mountain Lake, Va., on wild dewberry.
37.—Spores collected at French Creek, W. Va., on wild dewberry.
38.—Spores collected at Blacksburg, Va., on cultivated blackberry.
39.—Spores collected at Blacksburg, Va., on cultivated blackberry, variety Mersereau.
40.—Spores collected at Vienna, Va., on cultivated blackberry.
41.—Spores collected at Ithaca, N. Y., on wild blackberry.
42.—Spores collected at Blacksburg, Va., on cultivated blackberry, variety Ancient Britain.
43.—Spores collected at Janassee Junction, Ga., on wild blackberry.

44.—Spores collected at Thunderbolt, Ga., on *R. procumbens*.
45.—Spores collected at Hyattsville, Md., on wild dewberry.
46.—Spores collected at Hyattsville, Md., on wild blackberry.
47.—Spores collected at Blacksburg, Va., on cultivated blackberry.
48.—Spores collected at Vienna, Va., on wild blackberry.
49.—Spores collected at Willard, N. C., on wild blackberry.
50.—Spores collected at Connellsville, Pa., on wild blackberry.
51.—Spores collected at Auburn, Ala., on wild blackberry.
52.—Spores collected at Butte Creek Canyon, Calif., on *R. ursinus*.
53.—Spores collected at Stark, Fla., on wild blackberry.
54.—Spores collected at Blacksburg, Va., on wild blackberry.
55.—Spores collected at Thunderbolt, Ga., on *R. hispidus*.
56.—Spores collected at Hyattsville, Md., on wild blackberry.
57.—Spores collected at Blacksburg, Va., on wild blackberry.
58.—Spores collected at Hammond, La., on wild blackberry.
59.—Spores collected at Orlando, W. Va., on wild blackberry.
60.—Spores collected at Ithaca, N. Y., on wild blackberry.
61.—Spores collected at Blacksburg, Va., on wild blackberry.
62.—Spores collected at West Falls Church, Va., on cultivated blackberry.
63.—Spores collected at Arlington, Va., on wild blackberry.
64.—Spores collected at Arlington, Va., on wild blackberry.
65.—Spores collected at French Creek, W. Va. on wild blackberry.

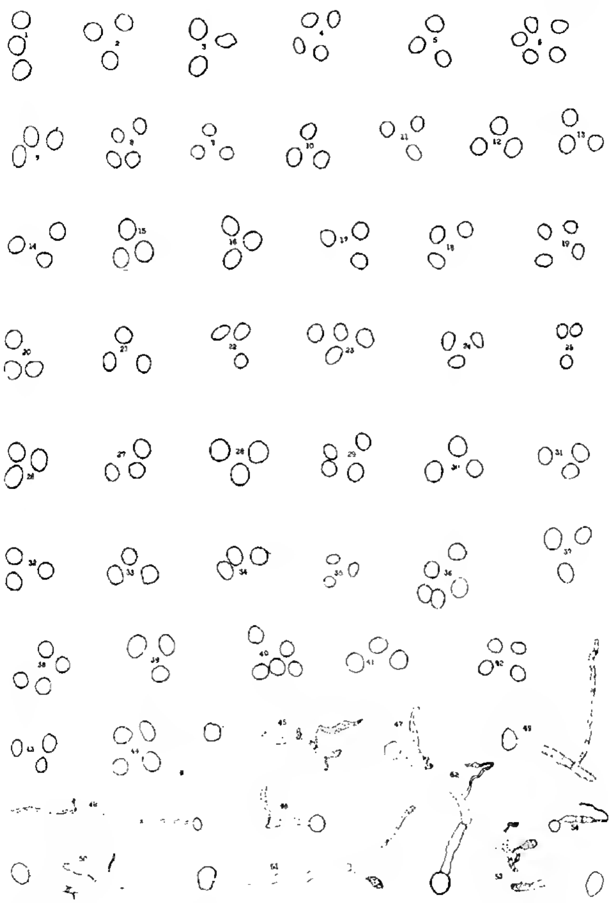

PLATE 94

Gymnoconia interstitialis. $\times 100$, except figures 48 and 54, which are $\times 53\frac{1}{3}$.

1.—Spores collected at Madrid, Me., on wild blackberry.
2.—Spores collected at French Creek, W. Va., on wild black raspberry.
3.—Spores collected at Glen, N. H., on *Rubus nigrobaccus.*
4.—Spores collected at West Falls Church, Va., on cultivated black raspberry.
5.—Spores collected at Vienna, Va., on cultivated black raspberry.
6.—Spores collected at Old Forge, N. Y., on *R. canadensis.*
7.—Spores collected at Glen, N. H., on wild blackberry.
8.—Spores collected at Mountain Lake, Va., on *R. alleghaniensis.*
9.—Spores collected at West Falls Church, Va., ou cultivated black raspberry.
10.—Spores collected at Portland, Me., on *R. canadensis.*
11.—Spores collected at Madison, Wis., on wild blackberry.
12.—Spores collected at Phillips, Me., on wild blackberry.
13.—Spores collected at Portland, Me., on *R. canadensis.*
14.—Spores collected at East Lansing, Mich., on wild blackberry.
15.—Spores collected at Sebago Lake, Me., on *R. triflorus.*
16.—Spores collected in Michigan on wild blackberry.
17.—Spores collected at Old Forge, N. Y., on *R. canadensis.*
18.—Spores collected at Madison, Wis., on wild blackberry.
19.—Spores collected at Old Forge, N. Y., on *R. canadensis.*
20.—Spores collected at West Falls Church, Va., on wild black raspberry.
21.—Spores collected at West Falls Church, Va., on cultivated black raspberry.
22.—Spores collected at West Falls Church, Va., on cultivated black raspberry.
23.—Spores collected at Glen, N. H., on *R. canadensis.*
24.—Spores collected at Mountain Lake, Va., on *R. alleghaniensis.*
25.—Spores collected at French Creek, W. Va., on black raspberry.
26.—Spores collected at Portland, Me., on *R. canadensis.*
27.—Spores collected at Smugglers Notch, Vt., on *R. strigosus.*
28.—Spores collected at Madrid, Me., on wild blackberry.
29.—Spores collected at Old Forge, N. Y., on *R. canadensis.*
30.—Spores collected at Smugglers Notch, Vt., on *R. canadensis.*
31.—Spores collected at Bancroft, Wis., on *R. hispidus.*
32.—Spores collected at Juliet, N. Y., on *R. canadensis.*
33.—Spores collected at French Creek, W. Va., on black raspberry.
34.—Spores collected at Old Forge, N. Y., on *R. canadensis.*
35.—Spores collected at Madison, Wis., on wild blackberry.
36.—Spores collected at Portland, Me., on *R. triflorus.*
37.—Spores collected at Sebago Lake, Me., on *R. triflorus.*
38.—Spores collected at Smugglers Notch, Vt., on *R. canadensis.*
39.—Spores collected at Sebago Lake, Me., on *R. triflorus.*
40.—Spores collected at Rouses Point, N. Y., on black raspberry.
41.—Spores collected at Sebago Lake, Me., on wild blackberry.
42.—Spores collected at Mountain Lake, Va., on *R. alleghaniensis.*
43.—Spores collected at Bound Brook, N. J., on black raspberry.
44.—Spores collected at Glen, N. H., on *R. canadensis.*
45.—Spores collected at Glen, N. H., on *R. canadensis.*
46.—Spores collected at Chain Bridge, near Washington, D. C., on black raspberry.
47.—Spores collected at Chain Bridge, near Washington, D. C., on black raspberry.
48.—Spores collected at Chain Bridge, near Washington, D. C., on black raspberry.
49.—Spores collected at Glen, N. H., on *R. canadensis.*
50.—Spores collected at Glen, N. H., on *R. canadensis.*
51.—Spores collected at Glen, N. H., on *R. canadensis.*
52.—Spores collected at West Falls Church, Va., on black raspberry.
53.—Spores collected at Vienna, Va., on black raspberry.

GERM-FREE FILTRATES AS ANTIGENS IN THE COMPLEMENT-FIXATION TEST

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In the production of germ-free blackleg filtrates, to insure uniformly good results it is of prime importance to check or control properly each lot of culture flasks, so as to know definitely that the blackleg organism alone has been growing therein. After the culture flasks have been inoculated and have been incubated for from six to nine days it is quite simple to remove one or more cubic centimeters of the culture and test it for the presence or absence of aerobic organisms. The detection of foreign anaerobes, however, should any be present, is not at all a simple procedure. Moreover, it would be quite impracticable to resort to the complicated process of anaerobic plating or fishing in the search of contaminating anaerobic microorganisms as a routine procedure with each lot of culture flasks. For this reason serological studies were made with pure germ-free blackleg filtrates to ascertain whether they would act as antigens in the complement-fixation test and, if so, what range of specificity could be obtained therewith, using the results as an index to what a satisfactorily produced product should possess.

Accordingly, blackleg filtrates were prepared, and a horse was repeatedly injected with them at intervals extending over a period of approximately three months. Blood serum drawn from this animal constituted the positive or immune serum. Antigenic titrations were then made, using 0.2 cc. of positive and 0.2 cc. of negative (normal horse) serum; and grading amounts of the germ-free filtrate were added as the antigen. The titration given in Table I will exemplify the character of reaction that has been obtained.

When the filtrate is concentrated over sulphuric acid in vacuo to one-half or one-third its original volume, the antigenic unit and the anticomplementary dose are reduced in the same ratio.

So far as the writer is able to learn by search through the literature, the use of a germ-free filtrate as an antigen in the complement-fixation test is an entirely new phenomenon; and it promises to serve a very important rôle in the separation and differentiation of the spore-bearing anaerobes. With this purpose in mind it is contemplated to parallel this reaction with the other pathogenic spore-bearing anaerobes as *Bacillus edematiens*, vibrion septique, *B. tetanus*, *B. botulinus*, etc.; and evidence of the feasibility of doing this is shown in the tests already made with

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B. botulinus filtrate. Good fixations were obtained by using *B. botulinus* (type B) filtrate with *B. botulinus* (type B) immune serum, but type B filtrate and type A serum would not produce a fixation, nor would type A filtrate produce a fixation with type B serum. Considering that type B immune serum does not protect guinea pigs against type A filtrate, which contains the type A toxin, and vice versa, the absence of fixation when using one type of serum and the other type of filtrate as antigen is quite important from a differential standpoint and also serves to indicate the specificity of the reaction obtainable by this method.

TABLE I.—*Titration of germ-free blackleg filtrate antigen*

Tube No.	Serum.		Physiological salt solution.	Antigen.[c]	Complement.		Hemolytic rabbit serum and sheep corpuscle.[d]	Result.[e]
	Positive.[a]	Negative.[b]						
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*		*Cc.*	
1	0.2	....	2	0.05	1	Incubated 1 hour at 37° C.	2	+
1	.2	....	2	.1	1		2	+
3	.2	....	2	.2	1		2	+
4	.2	....	2	.3	1		2	+
5	.2	....	2	.4	1		2	+
6	.2	....	2	.5	1		2	+
7	.2	....	2	.6	1		2	+
8	.2	....	2	.7	1		2	+
9	.2	....	2	.8	1		2	+
10	.2	....	2	1.0	1		2	+
11	.2	....	2	2.0	1		2	+
12	.2	....	2	....	1		2	−
1	....	0.2	2	.05	1		2	−
2	....	.2	2	.1	1		2	−
3	....	.2	2	.2	1		2	−
4	....	.2	2	.3	1		2	−
5	....	.2	2	.4	1		2	−
6	....	.2	2	.5	1		2	−
7	....	.2	2	.6	1		2	−
8	....	.2	2	.7	1		2	±
9	....	.2	2	.8	1		2	+
10	....	.2	2	1.0	1		2	+
11	....	.2	2	2.0	1		2	+
12	....	.2	2	....	1		2	−
13	....	.2	2	....	1		2	−
14	....	.2	2	....	....		2	+

[a] Horse serum hyperimmunized to germ-free blackleg filtrate.
[b] Normal horse serum.
[c] Germ-free blackleg filtrate.
[d] Hemolytic system employed consisted of a 3 per cent suspension of sheep red cells, 2½ units of hemolytic amboceptor, and 1½ units of complement, the latter being titrated against the amboceptor and sheep cells.
[e] + indicates complete inhibition of hemolysis; ±, partial inhibition of hemolysis; and −, no inhibition of hemolysis.

Since a blackleg filtrate produced from a pure culture of *Bacillus chauveaui* and grown under favorable conditions will possess antigenic value in the quantities shown in the preceding table, if a filtrate were encountered that failed to approximate such a titre then only would it

seem necessary to resort to the anaerobic cultural examination of the culture flasks. Calves inoculated with blackleg filtrate showing a satisfactory antigenic value were rendered sufficiently immune, after a period of three to four weeks, to withstand intramuscular injections of 100 to 200 mgm. of virulent blackleg muscle powder, a quantity sufficient to kill unvaccinated calves in two to three days.

Failure of a blackleg filtrate to possess an antigenic titre of from 1/10 to 1/20 the anticomplementary dose should arouse the suspicion that the blackleg organism did not grow under favorable conditions, that some contamination is present, or that the organism being used was not the blackleg organism at all.

CONCLUSION

From the data at hand it can be said that—

(1) A blackleg filtrate produced under favorable conditions will possess a distinct antigenic value demonstrable by the complement-fixation test.

(2) Those blackleg filtrates that conferred a solid immunity on calves were found to possess a high antigenic titre.

(3) The complement-fixation reaction should be of much value as a laboratory control test to determine whether the filtrate has been produced under conditions favorable to the blackleg organism or whether the blackleg organism has been supplanted in part or wholly by contaminating anaerobic microorganisms.

(4) Botulinus filtrate also acts as an antigen in the complement-fixation test when type B serum is used with type B filtrate but fails to cause fixation when one type of serum is used with the other type of filtrate as antigen.

(5) The phenomenon of germ-free filtrates acting as antigens in the complement-fixation test is new and promises to play an important part in the differentiation of the spore-bearing anaerobes, more especially those having closely similar cultural characteristics.

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MOSAIC DISEASE OF CORN [1]

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DISTRIBUTION

In connection with an investigation of the mosaic disease of sugar cane, a similar disease of corn has been observed by the writer on several occasions in widely separated regions.[2] On April 18, 1919, corn of an unknown variety was seen to be affected with typical mosaic symptoms in a field just west of Peñuelas, P. R. The percentage of affected plants was small, however, only 20 individuals being found in the field of some 5 acres. The corn averaged about 24 inches in height at this time and was planted between rows of sugar-cane stubble which had not been completely killed out in preparing the land for the corn. All the sugar cane was affected with mosaic. In July, 1919, corn of the White Creole variety was seen at the Sugar Experiment Station, New Orleans, La., in which the same condition was apparent. This corn was more than half grown, and the typical streaking of the leaves was somewhat obscured by certain leafspot diseases, among them the leafspot caused by *Physoderma zeae-maydis*, by which the corn was severely attacked. About 10 per cent of the plants in the field were affected with mosaic. In adjoining fields of sugar cane nearly 100 per cent of the plants were affected with the sugar-cane mosaic. In 1920 corn of the same variety was examined early in the season, and a much more serious infestation was found. The corn had been planted following sugar cane, and occasional diseased stools of the latter not killed by the plow were found all through the corn field. More than 30 per cent of the corn plants were affected. The cases were more abundant in the vicinity of the sugar-cane stools referred to above, but cases could be found many rods from any living cane. Of course, it is possible that a stool of cane had sprouted between the rows in such a situation and later had been killed by the cultivator. In May, 1920, identical cases of mosaic were seen in a field of corn near Cairo, Ga. As in the cases reported previously, a neighboring field of sugar cane was slightly infested with mosaic.

[1] The study of this disease was undertaken on account of its relation to sugar-cane mosaic.
[2] Brandes, E. W. THE MOSAIC DISEASE OF SUGAR CANE AND OTHER GRASSES. U. S. Dept. Agr. Bul. 829, 26 p., 5 fig., 1 col. pl. 1919.

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Diseases of corn bearing a decided resemblance to the one in question have been reported from other countries. Dr. H. L. Lyon states [1] that in the Hawaiian Islands a disease of corn which resembles sugar-cane mosaic is very serious. William H. Weston [2] describes a disease of corn in Guam which may be identical with the one under discussion. He mentions yellowing and dwarfing among the symptoms and states that the leaves exhibited mottling and striping.

VARIETAL SUSCEPTIBILITY

Just enough work has been done on varietal susceptibility to prove that all varieties of corn do not respond in the same way. The writer has never seen such excessive injury as that described for the unknown variety in Guam by Weston. In Louisiana the injury to corn of the White Creole variety, while marked in some individuals, was not excessive, excepting when the plants were infected early in the spring. The variety U. S. Select No. 182 is very susceptible to mosaic, but is not especially injured by it. Golden Bantam sweetcorn could not be infected in the greenhouse by methods which were successful with U. S. Select No. 182. Golden Bantam was planted unprotected in a greenhouse with hundreds of infected sugar-cane and sorghum plants. The corn aphis quickly migrated to the young corn plants from diseased sorghum in great numbers, but no cases appeared among the Golden Bantam seedlings. It seems probable that this variety is immune.

IMPORTANCE

No figures are available on the amount of loss sustained on account of injury to corn. The writer is inclined to believe that in this country no great damage has been done thus far. Probably the disease was introduced on sugar cane within comparatively recent years, in which case it may become more important in the future. At present, however, our chief concern is with its relation to the sugar-cane crop. Corn is almost invariably used in the rotation on sugar-cane land, so that no plantation is ever without corn in some of its fields. This means, of course, that the possibility for spread of the disease is greatly increased. Overwintering by the virus has been demonstrated only in the vegetative portions of the sugar-cane plant, but the existence of other graminaceous hosts certainly complicates the problem of control.

SYMPTOMS

In corn as in sugar cane the most conspicuous symptom of mosaic is the streaked and irregularly mottled appearance of the leaves. In corn, however, the lower, older leaves have a greater tendency to resume their normal color, so that it is sometimes difficult to demonstrate the

[1] In verbal communication, January, 1920.

[2] WESTON, W. H. REPORT ON THE PLANT DISEASE SITUATION IN GUAM. Guam Agr. Exp. Sta. Rpt. 1917, p. 45–62. 1918.

mosaic patterns in such leaves. In the youngest leaves, either the normal dark green or the pallid, affected tissue may predominate in a given specimen, but the latter condition is most frequently met with. In such cases the areas which remain normal are in the shape of broken or interrupted streaks or lines extending in the general direction of the long axis of the leaf (Pl. 95), and the contrast in color between these areas and the surrounding pallid areas is very decided. The streaks vary greatly in size, ranging from mere points to elongated "islands" of dark green 2 or 3 cm. or more long and several millimeters wide. The margins of such streaks may be straight or undulating. In most cases the mosaic pattern is more prominent at the base of the leaf, where it diverges from the leaf sheath. Where the normal dark green is predominant, the light green, affected tissue appears usually as a very fine mottling or as irregular elongated streaks on the darker background. From the foregoing description it can be seen that the patterns vary considerably, and yet they have certain general characteristics which make it almost impossible to confuse this condition with any other affecting the leaves.

Infected plants are always lighter in color than healthy plants. When viewed from a distance such plants can be picked out with a fair degree of accuracy on this account. The top of the plant is especially pale, much more so than normal freshly unrolled young leaves. In some cases the color becomes decidedly yellow. In this connection it must be stated that the pallid color referred to heretofore as characteristic of the diseased areas is not a yellowish green but a lighter or more dilute tint of the normal green. In plants which become markedly yellow a decided stunting of the whole plant takes place. At no time has a case been observed to terminate fatally, but certainly considerable injury results from the lack of functioning chloroplastids, and where a large percentage of the plants are affected the loss due to decreased size of ears is appreciable. When infection takes place early in the growing season, partial or complete sterility of the ears results. This serious feature of the disease was first noticed in Louisiana in 1920. In May, 1920, the writer tagged 20 diseased and 10 healthy plants in a field of White Creole corn. The diseased and healthy plants were equally vigorous to all appearances at that time and were in the same rows, alternate diseased and healthy plants in the same row being selected as far as it was practicable. When the crop was harvested in August, 17 of the diseased plants were found to be completely sterile, while 3 of them had set a few scattered kernels. The 10 healthy plants were normal, excepting for slight corn earworm injury, and produced large well-filled ears (Pl. 96).

During the course of experiments in the greenhouse several cases of apparent recovery have been observed. Plants which became infected and exhibited the typical symptoms resumed their normal color after several weeks. These plants were held under observation until the ears were mature, but there was no recurrence of the mosaic symptoms.

This interesting behavior was also noted in stools of crabgrass (*Syntherisma sanguinalis*) and foxtail (*Chaetochloa lutescens*). There were no changes of growing conditions that could be correlated with these apparent recoveries. In this connection it may not be out of place to record that suckers from diseased stools of sugar cane and sorghum have been observed to come up with no sign of mosaic. These instances are by no means common, but several have been seen in both plants mentioned.

INSECT TRANSMISSION OF CORN MOSAIC

The manner in which corn mosaic is transmitted to healthy plants and the relation of this disease to mosaic in other grasses was demonstrated by the following experiments.

EXPERIMENT 1.—On March 12, 1920, 12 corn plants of the variety U. S. Select No. 182 were placed in each of two insect-proof cages. All of the plants were from the same lot of seed furnished by the Office of Cereal Investigations. The seed had been planted in one flat, and the seedlings were replanted in 5-inch pots on the date of removal to the cages. They were then 12 inches tall. About 12 individuals of *Aphis maydis* were carefully removed by means of a small camel's-hair brush from sorghum plants affected with mosaic to each corn seedling in one of the cages. The sorghum plants had been infected by aphids from mosaic sugar cane. Twelve aphids were transferred in the same way from healthy sorghum to each of the corn seedlings in the adjoining control cage. On March 28, 6 of the 12 corn seedlings in the first cage showed typical signs of mosaic in the two youngest leaves. On April 6, 8 of the plants, or 66⅔ per cent, were typical cases. The 12 control plants remained healthy up to the time of removal several weeks later.

EXPERIMENT 2.—On April 6, 1920, 20 corn seedlings, variety U. S. Select No. 182, in 5-inch pots were placed in each of two insect-proof cages in the greenhouse. Several specimens of *Aphis maydis* were transferred from infected corn plants to each corn seedling in the first cage. Aphids from healthy corn in another greenhouse were placed on each corn plant in the second control cage, which was used as a control. On May 4, 7 of the corn seedlings in the first cage were found to be infected. On May 28, 15 of the 20 plants were observed to be unmistakable cases. The aphids had increased enormously in both cages. Not a single case could be found in the control cage, nor had any appeared up to June 25, although the plants had been repotted twice and were approaching maturity.

These experiments demonstrate conclusively that provision is made for almost unlimited dispersal of the virus through the medium of the corn aphis. There is no reason for supposing that transmission in nature is limited to this insect or to this method. It is not yet known whether the virus can survive the winter in seed, but experiments are now under

way that may throw some light on this phase of the problem. It has been proved that the virus of corn mosaic is identical with that of sugar-cane and sorghum mosaic, so that even if it is found not to be seed-borne, perpetuation of the disease in the perennial grasses would explain its appearance on corn in the spring.

Artificial transmission of the disease by means of inoculation with expressed cell sap of affected plants has not been attempted for corn. This method has proved successful in sugar cane, however,[1] and there is little doubt that the infectious material is contained in the cell sap of corn. Just what this infectious material is can not be stated definitely, but the evidence points strongly toward a living organism. No evidence incompatible with this view has been put forward for any mosaic disease, excepting the failure to demonstrate any visible organism.

CONTROL

Control measures for this disease must be based fundamentally on the removal of sources of the inoculum. So far as is known the only sources of inoculum are the living host plants. Destruction of these plants, then, will effectively eradicate the disease from any region. Practically, the destruction of all affected host plants presents almost unsurmountable obstacles. An immense amount of sugar cane is now infected in the River District of Louisiana and in southern Georgia. Destruction of large numbers of plants by roguing or plowing up is viewed with great concern by the planters, most of whom oppose any plan to control the disease by eradication. The substitution of immune varieties of corn as well as cane does not offer any immediate solution, since the most susceptible varieties happen to be the ones most esteemed. Elimination of this disease is dependent upon the education of the planter to an understanding of its seriousness. When this is accomplished public sentiment will permit of the passage of compulsory roguing and quarantine laws, which will be necessary before any hope can be entertained of eliminating the disease.

[1] Brandes, E. W. Artificial and insect transmission of sugar-cane mosaic. *In* Jour. Agr. Research, v. 19, no. 3, p. 131–138. 1920. Literature cited, p. 138.

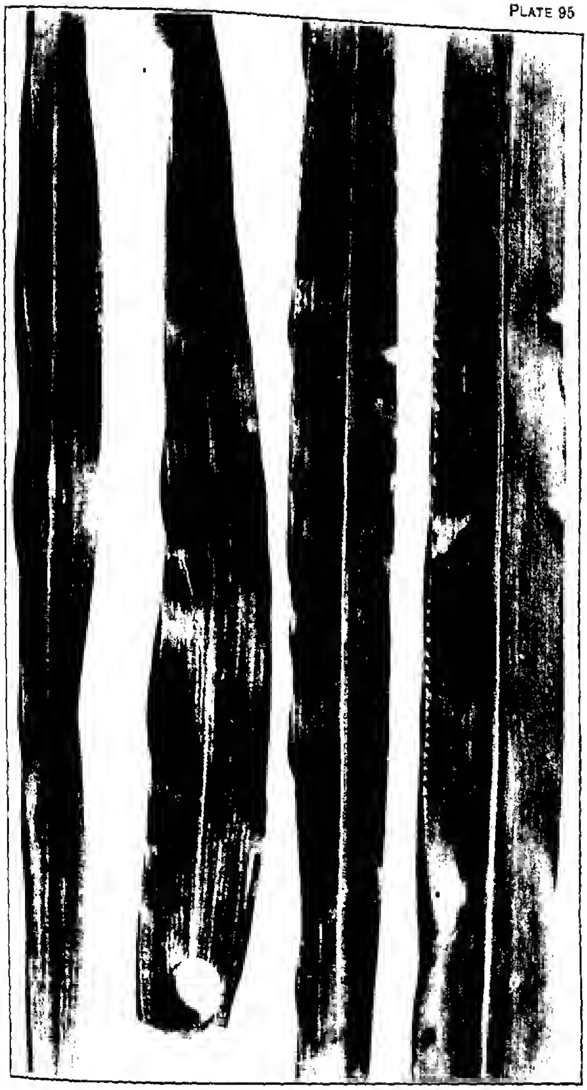
PLATE 95

Mosaic disease of corn:

The first leaf at the left shows the typical interrupted streaks of normal green in a pallid green background. The next leaf shows a more irregular, mottled pattern. In these specimens the normal green was similar to "nickel green" and the pallid green was similar to "rejame green" in Ridgeway.[1] The two leaves at the right are from a healthy plant and are presented for comparison.

[1] RIDGEWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

PLATE 95



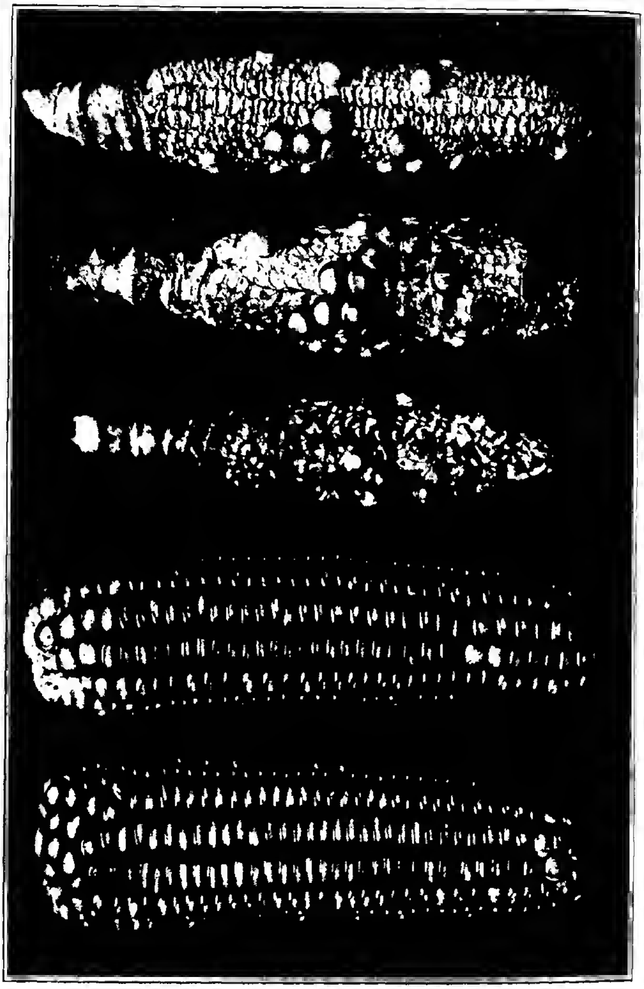


PLATE 96.

Mosaic disease of corn: Effect of early infection on the ear. White Creole variety.

The three ears at the top were produced by plants naturally infected in the field. In 17 out of 20 marked plants no kernels at all were developed.

The two lower ears are typical of all ears produced by healthy plants in the same row with the diseased plants.

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